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JUNTENDO MEDICAL JOURNAL

順天堂醫事雜誌

August 2022

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The History of *Juntendo Medical Journal*

This *Juntendo Medical Journal* has been published under the Japanese name *Juntendo Igaku* (順天堂医学) from 1964 to 2012. However, the origin of *Juntendo Medical Journal* dates back to the oldest medical journal in Japan, *Juntendo Iji Zasshi* (順天堂醫事雜誌), which had been published between 1875 and 1877 (total of 8 issues). Between 1885 and 1886, Juntendo issued a limited release of a research journal titled *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) for a total of 39 issues.

In 1887, *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) was published with the government's approval and we used to regard this as the first issue of *Juntendo Medical Journal*. Since then, *Juntendo Medical Journal* has undergone a series of name changes: *Juntendo Iji Kenkyukai Zasshi* (順天堂醫事研究会雜誌), *Juntendo Igaku Zasshi* (順天堂医学雑誌), and *Juntendo Igaku* (順天堂医学).

Now in commemoration of the 175th anniversary of Juntendo University, starting with the first volume issued in 2013 (Volume 59 Number 1), we return to *Juntendo Medical Journal*'s original Japanese title in 1875-*Juntendo Iji Zasshi* (順天堂醫事雜誌). We also reconsidered the numbering of the journal and set the first issue in 1875 as the initial publication of *Juntendo Medical Journal*. The Volume-Number counting system and the English name *Juntendo Medical Journal* started in 1955 from the January 10 issue. Although this is not our intension, we will retain the Volume-Number counting system to avoid confusion. However, Volume 59 Number 1 will be the 882nd issue, reflecting the sum of all issues to date: 8 issues of *Juntendo Iji Zasshi* (順天堂醫事雜誌), 39 issues of *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) (47 issues combined), and 834 issues from *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) in 1887 to the present.

出典：小川秀興（OGAWA Hideoki, M.D., Ph.D.）：順天堂醫事雜誌（Juntendo Medical Journal）2013；59：6-10.

本誌は昭和39年（1964年）から平成24年（2012年）末まで『順天堂医学』として刊行されてきた．しかし，その起源は明治8年（1875年）から10年（1877年）にかけて発刊された日本最古の医学誌『順天堂醫事雜誌』（計8巻）にある．さらに明治18年（1885年）から19年（1886年）まで，会員限定配本として順天堂醫事研究會の雑誌『報告』（計39集）が発行されている．

その後『順天堂醫事研究會報告』が明治20年（1887年）に官許を受けて公刊されたので，順天堂ではこれを通刊1号としてきた．以来，『順天堂醫事研究会雜誌』，『順天堂医学雑誌』，『順天堂医学』と名称を変更して刊行されてきた．

今般，順天堂が創立175周年を迎える平成25年（2013年）の59巻1号を期して，本来の名称である『順天堂醫事雜誌』と復刻し，その起源である明治8年（1875年）第1巻をもって創刊号（通刊第1号）とすることとした．従来の巻号と欧文誌名は，昭和30年（1955年）1月10日発行のものを1巻1号としており，欧文誌名もこれより付け始めたもので不本意であるが，混乱を避けるためにこれらを継承する．ただし，通刊数は明治8年（1875年）から19年（1886年）にかけて刊行された『順天堂醫事雜誌』8巻分と順天堂醫事研究會の雑誌『報告』39集，計47巻分を通巻834号に加え，59巻1号を通刊882号とした．

出典：小川鼎三，酒井シヅ：順天堂医学　1980；26：414-418.
小川秀興：順天堂醫事雜誌　2013；59：6-10.

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Contents

The Juntendo Medical Society

From the illustrator: In the middle of July, I went to Amami-Oshima Island for the first time, actually, in 30 years. The emerald green ocean I saw after a long time, white sand beaches, sound of crashing waves, and Pandanus fruits, which exactly reminded me of the world of paintings by Isson Tanaka, Japanese-style painter who had moved to the island. That was a very satisfying trip.

Special Reviews

Juntendo Medical Journal
2022. 68(4), 324-331

Fudan Zenshin, Kyumeikyukyu ; ~Now JIN again~

HIROSHI TANAKA

Department of Emergency and Disaster Medicine, Juntendo University Urayasu Hospital, Chiba, Japan

Summary: I graduated from Osaka University in 1982 and joined the Department of Traumatology, Osaka University Medical School. Patients with severe injuries and illnesses were brought in every day. Staff brushed up their skills on site, taught each other, and engaged in friendly competition for research. We had many frustrating moments when we could not save lives. Since then, the needs of emergency medicine have changed, and the scope of practice of emergency physicians has expanded to include pre-hospital emergency care, primary health care, intensive care, and disaster medicine. I was transferred to Juntendo University Urayasu Hospital in September 2007. Soon after my assignment, Urayasu Hospital was in the spotlight due to the emergency hospitalization of the All-Japan soccer coach and the Chinese frozen dumpling incident. It is said that emergency medicine is a mirror of society. I myself have experienced many disasters and incidents. It has been 15 years since I was assigned to this hospital, and I have 62 colleagues at Urayasu Hospital. They have all acquired various medical specialties, and some are emergency medicine specialists. In 2019, we hosted the 47th Annual Meeting of the Japanese Association for Acute Medicine. The theme of the conference is "Fudan Zenshin, Kyumeikyukyu (constant advancement, emergency medical services)". Emergency medical care is the starting point of "medicine" and is the ultimate source of life preservation for all citizens. We emergency physicians will continue to provide lifesaving medical care to patients without giving up until the very end, to keep the light of life from going out.

Key words: emergency medicine, pre-hospital emergency care, primary health care, intensive care, disaster medicine

Preface

I am pleased to announce that I will be retiring at the end of March 2022. I would like to express my sincere gratitude to Chairman Hideoki Ogawa and the many others who have guided and supported me throughout the years.

Before moving to Juntendo University

I graduated from Osaka University in 1982 and joined the Department of Traumatology, Osaka University Medical School because of my admiration for the work "Black Jack" by Osamu Tezuka, my senior in high school and college. At that time, patients with severe trauma, burns, poisoning, sepsis, and other serious illnesses were brought in every day. Staff brushed up their skills on site, taught each other, and engaged in friendly competition for research. We had many frustrating moments when we could not save lives. My mentor, Professor Tsuyoshi Sugimoto, often told me, "general surgeons should be ashamed of intraoperative death, and emergency surgeons should be ashamed when they miss the timing of surgery and let a patient die." Currently, the needs of emergency medicine have changed, and the scope of practice of emergency physicians has expanded to include pre-hospital emergency care, primary

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355th Triannual Meeting of the Juntendo Medical Society "Farewell Lectures of Retiring Professors"〔Held on Mar. 30, 2022〕
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emergency care, intensive care, and the field of disaster medicine, medical control in the region.

Frozen poisonous dumpling incident made in China

I was transferred to Juntendo University Urayasu Hospital in September 2007. Soon after my assignment, Urayasu Hospital was in the spotlight due to the emergency hospitalization of the All-Japan soccer coach and the Chinese frozen dumpling incident (1). An outbreak of food poisoning that affected at least ten people in various regions of Japan was traced to exposure to Chinese dumpling contaminated with the organophosphate insecticide Methamidophos. We experienced the most serious case, a five years old girl, who suffered coma. She presented with features of cholinergic overactivity and her serum cholinesterase activity was very low. We started intravenous treatment with pralidoxime iodide, atropine sulfate, and midazolam. Her symptoms improved gradually and she was discharged on day 25 without any sequelae. I and Dr Yuka Sumi got interviewed from a lot of media after her illness recovered (Figure 1). Dr Sumi, a member of us, is now working at the World Health Organization.

Emergency medicine is a mirror of society.

It is said that emergency medicine is a mirror of society. I myself have experienced many disasters and incidents (Table 1). In Osaka, I provided medical care for the Great Hanshin-Awaji Earthquake (2), the hemolytic uremic syndrome by O157 mass food poisoning, the Ikeda Elementary School child murder cases, and the JR Fukuchiyama train derailment accident. A catastrophic earthquake registering 7.2 on the Richter scale hit the southern part of Hyogo Prefecture including the city of Kobe, at 5:46 AM on January 17, 1995. The earthquake, subsequently knows as "Hanshin-Awaji," caused approximately 5,500 deaths and 41,000 injuries. Most of the dead were crushed or suffocated in collapsed dwellings. The number of partially destroyed dwellings reached approximately 100,000, and the number of those completely collapsed reached 93,000. Seven thousand one hundred dwellings burned down. We demonstrated morbidity and mortality of hospitalized patients within 14-days after the earthquake. Of the total 6,107 patients admitted to the 95 surveyed hospitals, 2,718 were injury patients, comprising 372 crush syndrome patients and 2,346 patients with other trauma. A total of 3,389 patients presented with illness. The mortality rates were 13.4% (50/372), 5.5% (128/2,346), and 10.3% (349/3,389) in crush syndrome, other trauma, and illness, respectively (Table 2). All patients admitted to surveyed hospitals are represented by the graph in Figure 2. Approximately 75% of trauma patients were hospitalized during the first 3 days. In contrast, the number of patients hospitalized for illness continued to increase during the entire 15-day period.

Figure 1 I and Dr Yuka Sumi got interviewed from a lot of media after the patient got well. Dr Sumi who is a member of us, and now she is working at the World Health Organization.

Table 1 Recent mass disaster and incident in Japan (2000~

VX nerve agent cases (1994)
the Great Hanshin-Awaji Earthquake (1995)
A series of sarin cases (1995)
O157 mass food poisoning (1995)
Wakayama Curry Incident (1998)
The Ikeda Elementary School child murder case (2001)
the JR Fukuchiyama train derailment accident (2005)
The Chinese frozen dumpling incident (2008)
The Akihabara indiscriminate murder case (2008)
The Great East Japan Earthquake (2011)
Mt Ontake eruption (2014)
Kinugawa flood (2015)
Kumamoto earthquake (2016)
Arson attack on an anime station in Kyoto (2019)
COVID-19 pandemic (2020)

Table 2 Patient Cllasfications and the Number of Related Deaths

	No. of Patients	No. of Deaths (%)
Crush syndrome	372	50 (13.4)
Other trauma	2346	128 (5.5)
Illness	3389	349 (10.3)
Total	6107	527 (8.6)

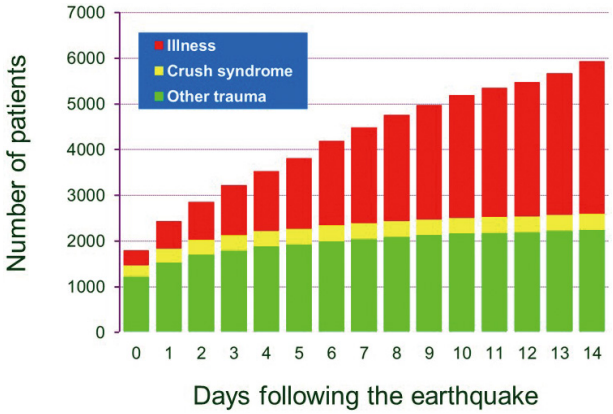


Figure 2 Cumulative patient census graph of all patients admitted to surveyed hospitals: green box, injury without crush syndrome; yellow box, crush syndrome; red box, illness.

The first case of the efficacy of steroid treatment for COVID-19.

After my transfer, I also experienced many disasters and incidents, including the Great East Japan Earthquake, and the coronavirus disease (COVID-19) pandemic, with which we are still battling. We reported the first case of the efficacy of steroid treatment for COVID-19 (3). A 67-year old man was transported to our hospital due to impaired consciousness and respiratory failure. After admission, tracheal aspirate of the patient was harvested, and it tested positive for severe acute respiratory syndrome coronavirus 2 nucleic acid. He required veno-venous extracorporeal membrane oxygenation (V-V ECMO) to sustain his oxygenation. However, his respiratory failure did not improve for 20 days. On day 20 of admission, we started to use i.v. steroid therapy. On day 23, lung opacity on the chest X-ray cleared and removed ECMO on day 27. We are successfully tapering steroids without serious adverse events, and he was removed from the ventilator on day 51 (Figure 3).

Our various research areas (from clinical research to basic one)

It has been 15 years since I was assigned to this hospital, and I have 62 colleagues at Urayasu Hospital. They have all acquired various medical specialties, and some are emergency medicine specialists. Eighteen have earned doctoral degrees in medicine and many have studied abroad at Harvard University and the Feinstein Institute in New York.

-Clinical research-

Our research areas are diverse (Table 3). In clinical research, studies on the emergency room included triage by rapid lactate measurement, relationship with dizziness and heart rate variability,

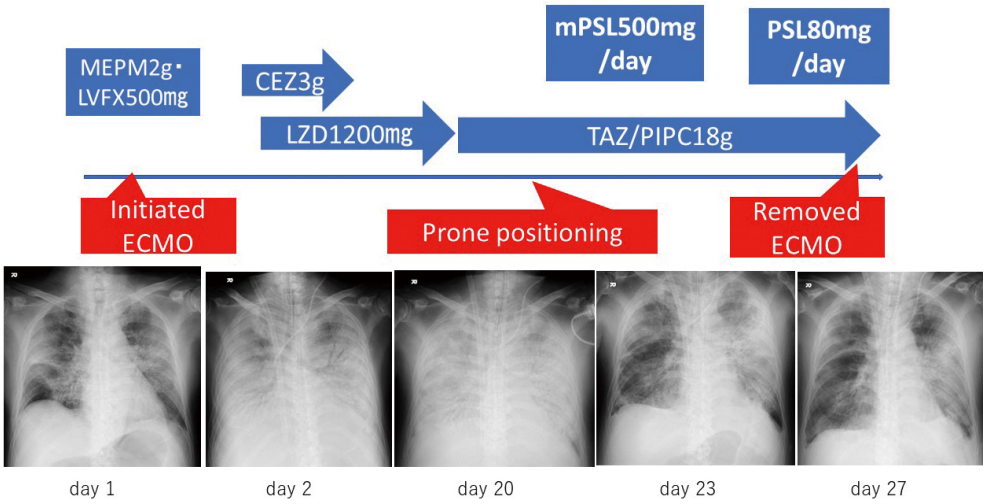


Figure 3 Chest X-ray images in time series of a 67-year old man during hospitalization for severe COVID-19-induced acute respiratory distress syndrome. Chest X-ray on day 1 shows bilateral basal consolidation with periphearal ground-glass opacity. Chest X-ray on day 2 shows bilateral pulmonary infiltrate mainly in hilar region. Chest X-ray on day 20 shows bilateral diffuse pulmonary infiltraes with air bronchogram. Chest X-ray on day 23 shows improved bilateral lung opacity (mainly in the right lobe of lungs). Chest X-ray on day 27 (extracorporeal membrane oxygenation removed).

Table 3 Our various research areas

Clinical research
Studies on ER science
Triage by rapid lactate measurement
Dizziness and heart rate variability
Registry studies in pediatric emergencies
Prognostic comparison of pediatric severe trauma
Epidemiological studies of critical ill pediatric pateient
Intensive care field
Systemic review on hyperosmotic infusion in severe head injury
Prognostic model for patients with cardiopulmonary arrest
Role of complement system in patients with multiple organ failure (KAKENHI Grant number 19H03764)
Disaster and Prehospital research
BCPs for disasters
Studies on securing intensive care staff during a pandemic
Studies on water supply in the earthquake
Effectiveness rapid response car
Establishment of an emergency medical care system in large theme park
Effectiveness of electronic triage during disasters (CREST)
Basic research
Neutrophil phenotype studies in sepsis
Dynamic of aged neutrophils
ICAM-1 positive neutrophils and NETs
Identification of low-density neutrophil
ATP targeting studies
Fluorescence imaging of ATP on the surface of neutrophils and in mitochondria
Neutrophil signaling transduction
Joint research course
Emergency AI Color Image Information Standardization Course

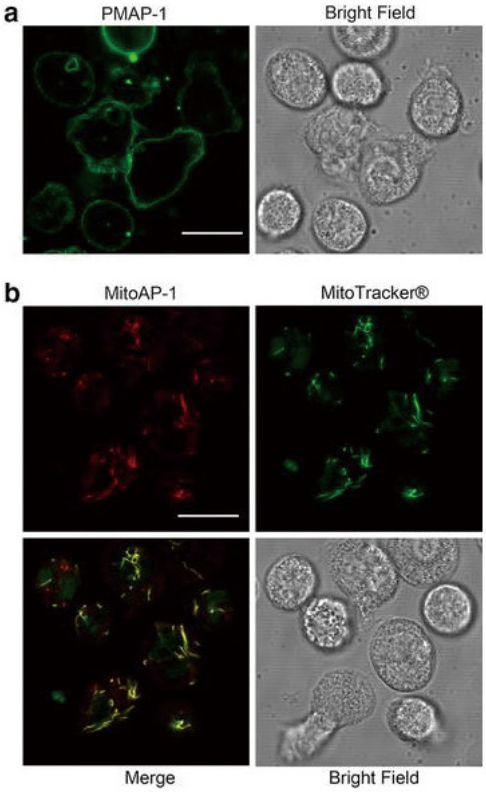
while registry studies in pediatric emergencies included prognostic comparisons of pediatric severe trauma, and epidemiological studies of critically ill pediatric patients. Clinical research in the field of intensive care includes systemic review on hyperosmotic infusion in patients with severe head trauma and the development of a prognostic model for patients with cardiopulmonary arrest (4).

-Disaster medicine and pre-hospital emergency medicine-

Studies in the field of disaster medicine and pre-hospital emergency medicine involves those on the establishment of business continuing plans (BCPs) for disasters. Other studies include research on the effectiveness of Rapid Response Car (5), the establishment of an emergency medical care system in large theme parks, and research on the effectiveness of electronic triage during disasters, which was adopted by CREST.

-Basic research (neutrophil phenotype and ATP)-

Basic research includes neutrophil phenotype studies during invasion (e.g., during sepsis) and studies targeting ATP. The former includes the dynamics of aged neutrophils, ICAM-1 positive neutrophils and NETs, and identification of low-density neutrophil subsets. In the research on neutrophil ATP, we conducted studies on fluorescence imaging of ATP on the surface of neutrophils and in mitochondria, and neutrophil signaling transduction (Figure 4) (6). Stimulation of the formyl peptide receptor was previously found to cause ATP release from PMNs through maxi-anion channels and PANX1 hemichannels. To confirm this finding, the median fluorescence intensity (MFI) of PMAP-1 on the plasma membrane of healthy control (HC) PMNs was quantified by flow cytometry after stimulation with the indicated concentrations of fMLP (Figure 5a). The MFI of PMAP-1 following fMLP stimulation increased in a dose-dependent manner. CD11b expression on the plasma



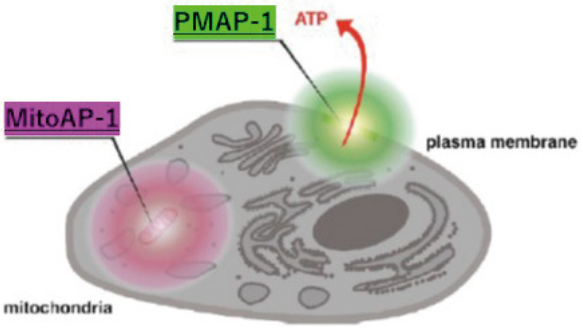


Figure 4 Confocal micrographs of human polymorphonuclear neutrophils (PMNs) stained with PMAP-1 and MitoAP-1.
a ATP on the plasma membrane was stained with PMAP-1. The *bright green* fluorescence observed on the plasma membrane of PMNs resulted from PMAP-1 conjugation to ATP (×100 oil objective, NA 1.4). *Scale bar* 10 μm.
b ATP in the mitochondria was stained with MitoAP-1 (*red*) and MitoTracker® Green FM (*green*), and they co-localized well (×100 oil objective, NA 1.4). Scale *bar* 10 μm

membrane of PMNs was also measured as a marker of PMN activation. Cell surface CD11b expression in PMNs stimulated with fMLP also increased in a dose-dependent manner (Figure 5b). Mitochondria are often referred to as the powerhouse of the cells, as they generate ATP by oxidative phosphorylation. To our knowledge, changes in mitochondrial ATP levels in PMNs have not been reported. The MFI of MitoAP-1 following fMLP stimulation decreased significantly in a dose-dependent manner (Figure 5c). The MFIs of PMAP-1 and MitoAP-1 in sepsis patients were evaluated by flow cytometry within 24 h after diagnosis of sepsis (days 0-1). Both values were significantly higher than those of the HC group (data not shown). CD11b expression on the plasma membrane was also upregulated in sepsis patients. Activated PMNs in sepsis patients appeared to cause a burst of extracellular ATP release and to increase ATP synthesis by oxidative phosphorylation in the mitochondria. The same experiments were conducted at days 3-4 as the patients' clinical conditions improved, which revealed that the MFIs of PMAP-1 and CD11b expression decreased significantly compared to those at days 0-1, whereas a high MitoAP-1 MFI was maintained. Namely, the temporal changes of ATP release and CD11b expression on the plasma membrane were similar, but ATP production showed distinct behavior in the mitochondria.

-Complement system in multiple organ failure-

Recently we are trying to elucidate the of complement system in patients with multiple organ failure by the grant for Japan Society for the Promotion of Science (JSPSI, KAKENHI Grant number 19H03764). Sepsis is a life-threatening emergency that occurs when the human body reacts in an extreme way to an infection, triggering a chain reaction that exacerbates the patient's condition. Almost any type of infection can lead to sepsis, although the most common infections typically start in the lung, urinary tract, skin or gastrointestinal tract. One of the main problems associated with sepsis is the multiple organ failure or dysfunction that it can lead to - it is this which most often leads to complication resulting in death. However, despite much research into this subject, the actual causes for multiple organ failure from sepsis (as well as severe trauma, burns and heat stroke) are not entirely clear. We have turned our attention to trying to ascertain the exact reasons why multiple organ failure occurs in sepsis patients. We have devel-

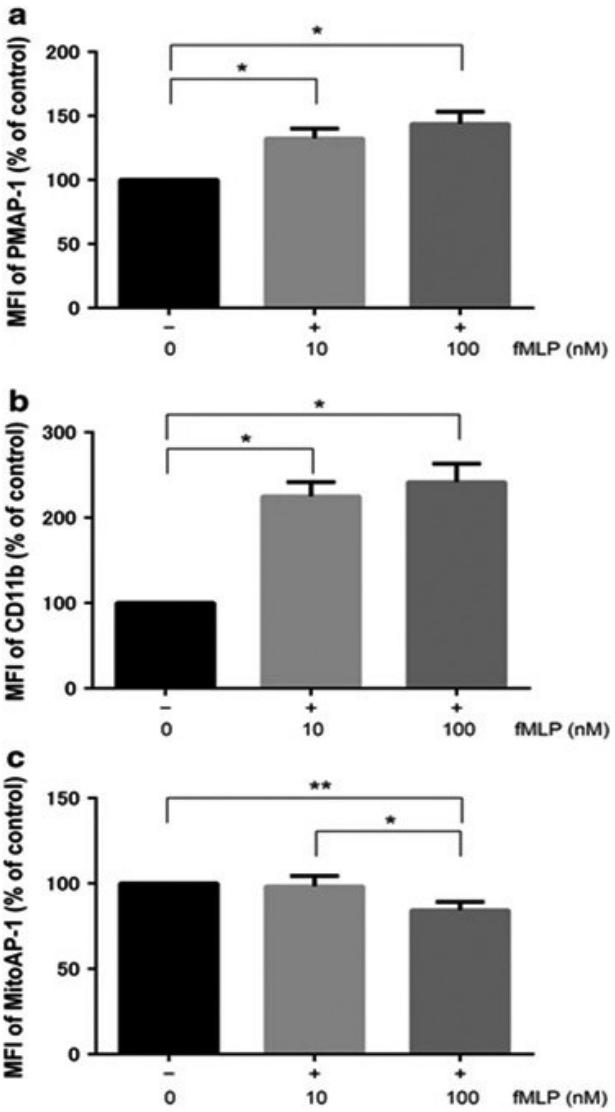


Figure 5 ATP level and CD11b expression after fMLP stimulation in healthy control subjects.
a Changes in the mean fluorescence intensity (MFI) of PMAP-1 after stimulation with the indicated fMLP concentrations; MFI values were normalized to those of controls (no fMLP) (n = 8 per group). The data shown are the mean ± SEM, and groups were compared with one-way ANOVA and Tukey's post hoc test (*$p < 0.01$).
b Changes in CD11b expression of polymorphonuclear neutrophils after fMLP stimulation; expression levels were normalized to those of controls (no fMLP) (n = 8 per group). The data shown are the mean ± SEM, and groups were compared with one-way ANOVA with Tukey's post hoc test (*$p < 0.01$). c Changes in MFI of MitoAP-1 after stimulation with the indicated fMLP concentrations; MFI values were normalized to those of controls (no fMLP) (n = 8 per group). The data shown are the mean ± SEM, and groups were compared with one-way ANOVA with Tukey's post hoc test (*$p < 0.01$, **$p < 0.05$)

oped a hypothesis that one of the causes for multiple organ failure in sepsis patients is complement activation. The complement system is a term denoting a series of more than 20 proteins that circulate in the blood and tissue fluids. Complement causes the killing of bacteria and the recycling of dead cells in the body and is essential to an effective immune response in the human body. Complement activation occurs during a range of alien invasions, such as viral infection, allergy, severe trauma, heat stroke and sepsis. In recent years, it has become clear that excessive complement activation can cause thrombotic microangiopathy (TMA), which is a condition that can lead to organ damage. Knowing this, we set about working to understand the extent to which complement activation is involved in multiple organ failure during biological infection. It is known that when the protein complement component 3 (C3) is activated by the immune system in response to infection, foreign objects or external stimulus, it becomes C3a and C3b, the latter of which binds to the surface of the cell membranes of microorganisms and reacts with factor B and factor D to form C3 converting enzymes. Moreover, when this particular pathway weakens the function of complement regulators in the body, it becomes amplified and becomes C5a and C5b which leads to the development of a variety of pathological conditions. We are therefore working on aspects of this knowledge with a view to determining whether uncontrolled complement activation occurs during biological infection and whether TMA is triggered, eventually leading to multiple organ failure. To achieve their aims, we have investigated the suppression of multiple organ failure in both clinical and basic research by studying the quantitative evaluation of the complement activation, its relationship with TMA, the relationship between complement activation and leukocyte/platelet and the control of complement activity. To perform the experiments, we used tools that are common to immunology, such as ELISA (enzymelinked immunosorbent assay) and FACS (flow cytometry) (7).

-A joint research course (Emergency AI Color Image Information Standardization Course)-

Additionally, a joint research course (Emergency AI Color Image Information Standardization Course) was established with Toppan Printing Co. Ltd since

last year. This study pursues the authenticity of color image data in the field of emergency medicine. Our goal is to build a platform for providing medical image information and to apply it clinically to emergency medical care settings using image information standardized by numerical color information.

The 47th Annual Meeting of the Japanese Association for Acute Medicine

In 2019, we hosted the 47th Annual Meeting of the Japanese Association for Acute Medicine (Figure 6). The theme of the conference is "Fudan Zenshin, Kyumeikyukyu; Now JIN again.". We have been continuously moving forward in acute medicine and critical care (Fudan Zenshin, Kyumeikyukyu), with Juntendo spirit JIN (humanity) that is now required again. Emergency medical care is the starting point of "medicine" and is the ultimate source of life preservation for all citizens. We emergency physicians will continue to provide lifesaving medical care to patients without giving up until the very end, to keep the light of life from going out.

Conclusion

Finally, I would like to thank all the medical staff and my family for their support. I would like to conclude my retirement address by wishing Juntendo's continued growth and good health to all those who are a part of Juntendo (Figure 7).

Figure 6 The 47th annual meeting of the Japanese Association for Acute Medicine, held at Octorber 2 to 4, 2019 in Tokyo.

Acknowledgments

I would like to thank all of the team members who have worked at the Department of Acute and Critical Care Medicine in Urayasu Hospital over the years, supporting a large number of activities.

Funding

No funding was received.

Author contributions

HT. wrote the manuscript.

Figure 7 Current medical staff in the Department of Emergency and Disaster Medicine, Urayasu Hospital.

Conflicts of interest statement

The author has no conflict of interest to disclose.

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Special Reviews

Juntendo Medical Journal
2022. 68(4), 332–338

My 42-year Experience in Radiation Oncology

KEISUKE SASAI[1, 2)]

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[2)] Department of Radiation Therapy, Misugikai Satou Hospital, Osaka, Japan

In the present review, I provide an overview of the development of radiation therapy and short history of the Department of Radiation Oncology, Juntendo University. I also emphasize the importance of radiation therapy as a major treatment modality for cancers.

Radiation therapy is a standard treatment for malignant tumors. It aims to deliver a sufficient radiation dose to a target volume to eradicate tumor cells or relieve symptoms of disease. Therapy can achieve good results in many types of cancers. Although radiation therapy sometimes causes undesirable adverse events, it is generally less invasive than other treatment modalities and does not alter the shape and function of healthy organs. When the author joined this field in 1981, radiation therapy techniques were highly primitive; however, during the past 42 years, treatment has advanced rapidly with the development of computer science, mechanical techniques and instrumentation. Currently, patients can be treated with precise radiation techniques, including intensity-modulated radiation therapy, image-guided radiation therapy, stereotactic irradiation, and brachytherapy. We also introduced a new treatment planning system that uses not only anatomical but also metabolic imaging, which permits correct delineation of the target volume. Therefore, it is crucial to stay up to date with advances and developments in rapidly emerging technologies to maintain high-quality treatment. The Department of Radiation Oncology at Juntendo University (Tokyo, Japan) is still small; however, it is gradually expanding and conducting research in both clinical and basic fields. It is the author's hope that many young investigators will join this field in the future.

Key words: radiation therapy, radiation oncology, image-guided radiation therapy, intensity modulated radiation therapy, biological target volume

Introduction

Radiation therapy is a standard treatment for malignant diseases. It is a treatment modality that aims to deliver a sufficient radiation dose to a target volume to eradicate tumor cells or relieve symptoms of disease.

In the United States, approximately one-half of patients with cancers undergo radiation therapy; however, only one-quarter of such patients are irradiated in Japan[1)]. There are several reasons for this low frequency of radiation therapy. For example, the number of cancers that are not candidates for radiation therapy, such as gastric malignancy, are higher than those in the United States or in European countries. Additionally, as a result of the destructive atomic bombings of Hiroshima and Nagasaki (Japan), and the Fukushima nuclear catastrophe, many Japanese generally fear radiation and radiotherapy.

In the present review, I provide an overview of the development of radiation therapy and short history of the Department of Radiation Oncology, Juntendo University (Tokyo, Japan). I also empha-

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size the importance of radiation therapy as a major treatment modality for cancers.

Developments in radiation therapy since 1981

When I joined this field in 1981, radiation therapy techniques were highly primitive. Virtually all institutions in Japan were equipped only with low-energy photon sources, such as cobalt-60 machines or low-energy medical linear accelerators (Linacs). Treatment was usually performed using a simple technique, such as two opposing anterior-posterior and posterior-anterior ports. The field was trimmed using one or two monoblocs fabricated from thick heavy metals. The radiation treatment field was determined using an X-ray simulator, which is a type of X-ray fluoroscopy specifically designed for radiation therapy treatment planning, and it has the same geometric arrangement as the treatment device. The field was determined based on anatomical landmarks, such as bones, which are visualized using X-rays. For example, radiation fields for uterine cervical cancer were determined at the upper end, between the 4th and 5th lumbar vertebrae, the lateral margin at 1.5 cm lateral to the inner margin of the iliac bone, and the lower margin at the level of the caudal end of the obturate foramen. However, some institutions did not have an X-ray simulator; as such, they had to use fluoroscopy dedicated to diagnostic use or simple X-ray photography. Low-energy photons cannot sufficiently penetrate to deep-seated areas of disease because they rapidly lose their energy along their track in the human body. Therefore, an extremely high dose was administered to the skin to treat deep-seated tumors, which sometimes caused severe side effects. The aforementioned factors, therefore, limited cure to only diseases located in superficial regions or easily approachable tumors, such as uterine cervical cancers in the early 1980s. As such, it was generally believed that radiation therapy was not a curative but a palliative method.

However, rapid advances in computer science and mechanical technologies have led to a revolution in the field of radiation oncology. Linacs with ultra-high-energy X-rays, which can easily reach deep-seated lesions, are now commercially available. In the final decade of the 20th century, many new techniques emerged. If sufficient radiation doses could be delivered to the target volume, desirable performance in treatment was achieved. Stereotactic radiosurgery uses three-dimensional images and focuses multidirectional beams on a small target. The treatment was first applied to intracranial lesions, then gradually extended to extracranial diseases (Figure 1) and has yielded a

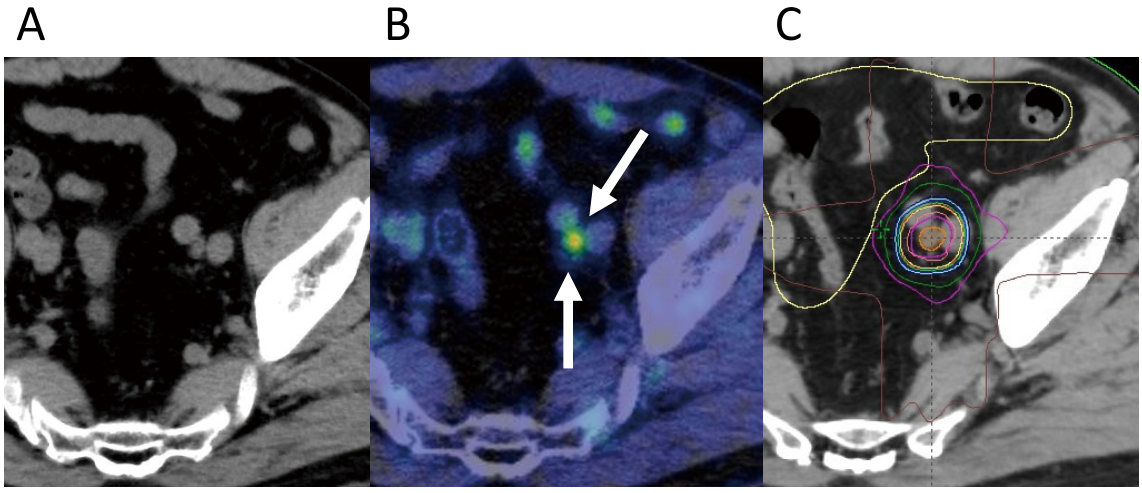

Figure 1 A male patient with prostate cancer underwent salvage radiation therapy for biochemical recurrence after surgical resection. However, his prostate-specific antigen levels gradually increased after five years. Computed tomography (CT) could not detect any recurrent lesions (A); however, ^{18}F- fluorodeoxyglucose positron emission tomography combined with CT clearly revealed suspected lymph node disease (B, indicated by white arrows). This lesion was treated using stereotactic radiation therapy using a total dose of 50 Gy in 10 fractions. Dose distributions of the treatments (C). Prostate-specific antigen levels dramatically decreased after treatment.

very high frequency of disease eradication. From Juntendo University, Naoi and colleagues were pioneers in this field in Japan and published high-quality reports[2,3].

Takahashi and colleagues proposed "conformation radiation therapy" in the 1960s[4,5]. This is a type of rotational radiation therapy, in which the tumor(s) is irradiated in a 360°direction, and the beams are trimmed to conform to the shape of the target volume during irradiation. However, the technique was not very popular until the 1990s because it was very complicated and there was no way to obtain trans-axial images of the body except by using Takahashi's rotation tomograms. In the 1990s, major instrument manufacturers equipped their Linacs with a multi-leaf collimator (Figure 2), which can easily shape the radiation field to conform to the target. Computed tomography (CT), which was introduced in the early 1970s, has also advanced to provide sufficient image quality for treatment planning. Since then, a new technique, known as "conformal radiation therapy", in which a target is irradiated by conformal beams from several fixed directions, has emerged and is widely used. At the turn of the new millennium, a more sophisticated treatment technique, known as intensity-modulated radiation therapy (IMRT), has been introduced in this field[6,7], with developments in this technology advancing virtually every year. It can be used to treat patients using an acceptable dose distribution (Figure 3).

Another advance in treatment is the introduction of image-guided radiation therapy (IGRT)[8]. IMRT has a steep fall-off of the radiation dose at the edge of the target volume. If the position of the target volume differs in a radiation session from the planning CT, the volume receives a lower dose than the plan prescribes. To overcome this problem, the position of the target is monitored before or during each treatment session using imaging modalities such as CT, magnetic resonance imaging (MRI), and/or ultrasound. Furthermore, it is possible to detect the movement of the target during irradiation. At Juntendo University Hospital in Hongo, tumor movement was tracked during a treatment session using the SyncTrax system

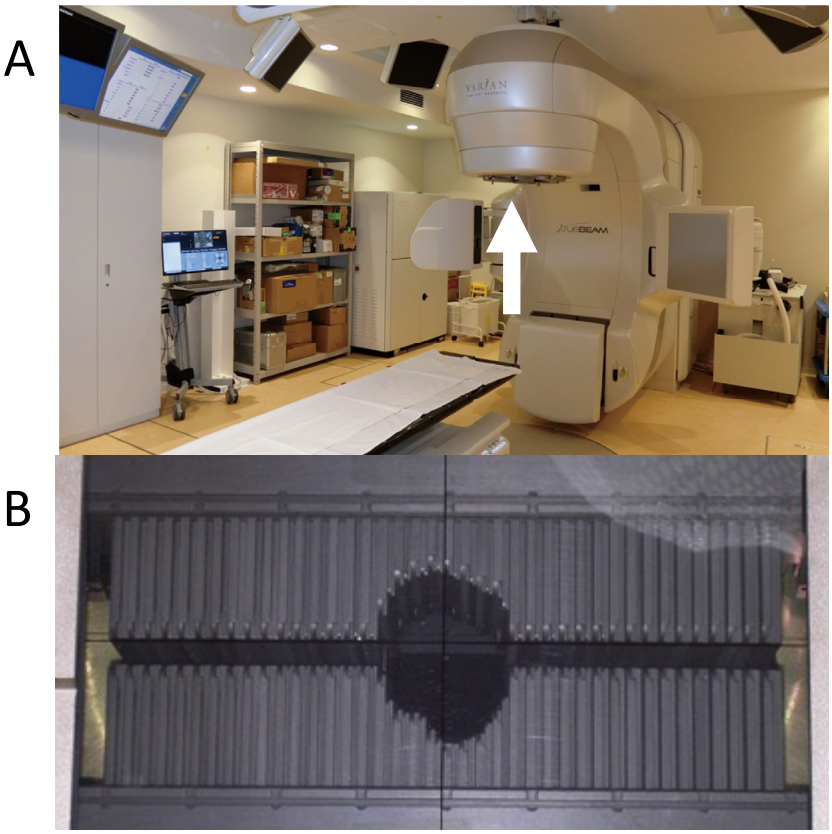

Figure 2 A medical linear accelerator (Linac) at the Juntendo University Hospital (Tokyo, Japan) (A) and multileaf collimator (B) placed at the aperture of the device, indicated by the white arrow.

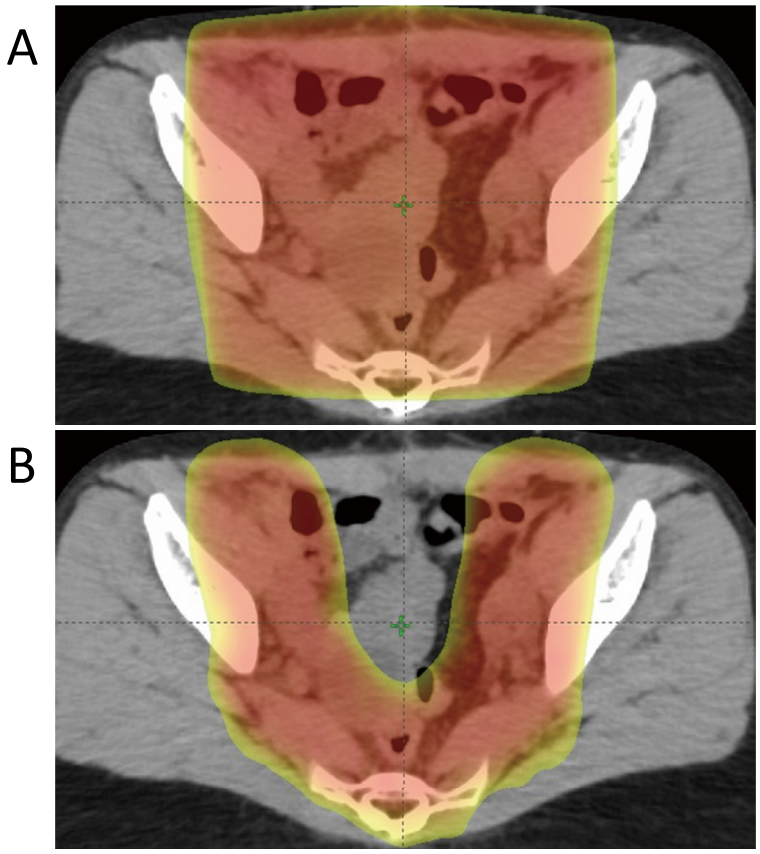

Figure 3 Dose distributions in conventional conformal radiation therapy (A) and intensity modulation radiotherapy (B).

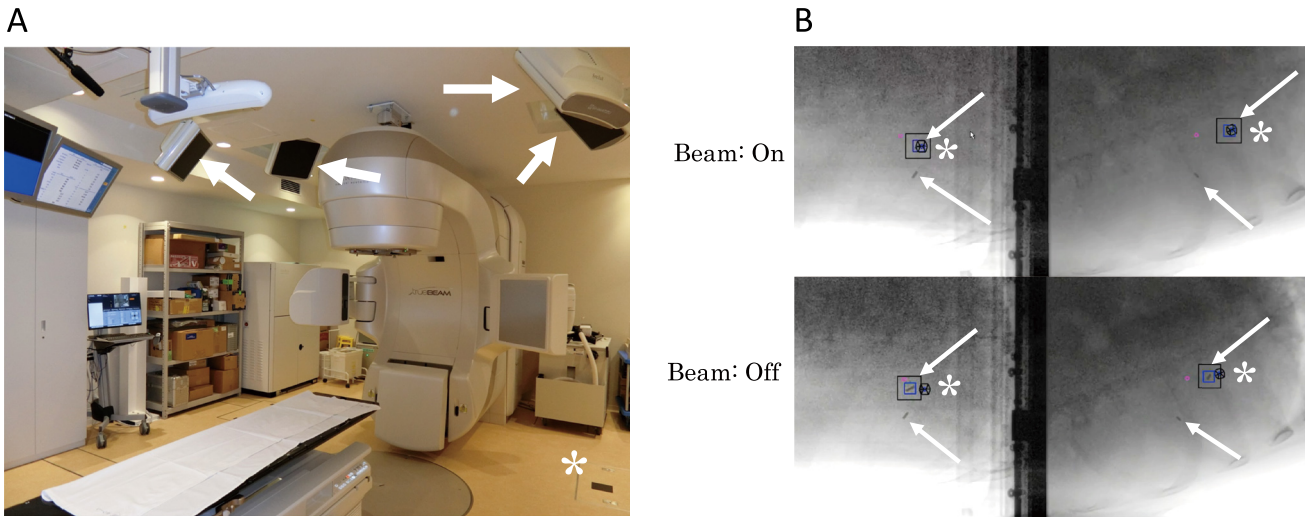

Figure 4 (A) The tracking system on a medical linear accelerator (Linac). The white arrows indicate X-ray detecting boards and the asterisk indicates one of four X-ray sources placed under the floor.
(B) We usually use two sets of X-ray systems to tract a gold fiducial marker placed near the target volume. While the marker is out of position, the Linac beam is off. If the marker moves into the predefined position on images from both directions, the radiation beam is on.

(Shimadzu, Kyoto, Japan) (Figure 4), a real tract radiation system[9]. It is combined with stereotactic radiation therapy to mainly treat lung or liver cancers. This technique, however, has drawbacks, including exposure to X-rays and the visualization of only the fiducial markers inserted near the target volume. A newly emerged MRI-guided treatment technique can detect the movement of

the target itself without any harmful effects[10], and may be a future direction of research and therapeutics.

In 2000, Ling et al. proposed a concept known as "biological target volume"[11]. As mentioned above, multiple modalities can be used to precisely irradiate the target volume. However, defining the target volume, which is usually based on anatomical images, remains a problem. Ling et al. proposed the use of biological and mechanistic data to delineate target volumes. Biological images broadly include metabolic, biochemical, physiological, functional, molecular, genotypic, and phenotypic. Although positron emission tomography (PET) using ^{18}F- fluorodeoxyglucose (FDG) is available for this purpose at Juntendo (Figure 1), other imaging modalities, such as ^{18}F-misonidazole PET for hypoxic cells, have been tested at other institutions[12, 13]. With advances in diagnostic and imaging modalities, functional imaging is anticipated to be introduced in this field in the future.

Lack of qualified personnel in this field is a major issue in Japan. There were only 899 certified radiation oncologists (ROs), 1213.9 full-time equivalent (FTE) ROs, and 295.7 FTE medical physicists, despite 846 institutions treating patients using radiotherapy in 2015[1]. Juntendo also contends with this problem, and will be addressed later.

Radiation therapy as a standard treatment for cancer

As shown in Table 1, radiation therapy can achieve good treatment results. Although it sometimes causes undesirable adverse events, it is generally less invasive than other modalities, and can preserve the shapes and functions of healthy organs. Therefore, it is regarded to be standard treatment for many types of malignant diseases and appears in domestic and international treatment guidelines for cancers. The therapy can be used not only as monotherapy, but can also be combined with other methods, including surgery, chemotherapy, and/or immunotherapy.

Radiation therapy at Juntendo University

When I was appointed to Juntendo University in 2000, the Radiation Oncology Division was a small part of the Department of Radiology. There was only one other RO with the exception of myself, although the individual was young and uncertified. Juntendo University had only two old-type Linacs (one at Juntendo University Hospital and another at Urayasu Hospital, Chiba, Japan).

The term "radiology" does not necessarily refer to radiation oncology (therapy) but refers to diagnostic radiology in the United States and major European countries. Because cancer is a leading cause of death, the Japanese government created the "Basic Plan to Promote Cancer Control Programs" based on the Cancer Control Act. One of the major policies is to promote radiation therapy, with the government encouraging each medical school to establish a radiation oncology department. In 2013, Juntendo University also established the

Table 1 Radiation therapy results for representative diseases at Juntendo University Hospital

Disease		Local control (%)	5-year (%)	7-year (%)	10-year (%)
Prostatic cancer	Low risk		100*	100*	100*
	Intermediate risk		95.1*	92.0*	89.2*
	High risk		96.1*	93.2*	85.7*
	Salvage radiation therapy		83.6*	76.7*	
Breast cancer	Conventional fractionation		98.7**		95.9**
	Accelerated fractionation		98.3**		95.3**
Uterine cervical cancer ***	Stage I		100***		
	Stage II		84***		
	Stage III		78***		
	Stage IVA		40***		
SRT for lung cancer		96			

*Biochemical relapse free rate, **the ipsilateral breast tumor control rate, Yoshida-Ichikawa Y et al. Breast Cancer 2021 Jan; 28(1): 92-98
***Overall survival, SRT: stereotactic radiation therapy

Department of Radiation Oncology, and I was appointed to be the first Chair. Juntendo Hospital is now equipped with three cutting-edge Linacs and a remote after-loading brachytherapy system. These facilities permit the treatment of virtually all cancer types that are suitable candidates for radiation therapy. Table 1 summarizes the results of radiation therapy for major diseases in our department[14]. Generally, these values were better than expected. Other affiliated hospitals have also been equipped with new instruments, including Shizuoka Hospital (Shizuoka, Japan), with one; Urayasu Hospital, with two, and Nerima Hospital (Tokyo, Japan), with one (Table 2). Tables 2 and 3 summarize the changes in the radiation therapy facilities in the Juntendo University group and the number of patients treated at Juntendo Hospital, Hongo. Although there are few members in the Department of Radiation Oncology, the number has gradually increased to 11 ROs and 3 physicists.

Research at the Department of Radiation Oncology

Research themes at the Department of Radiation Oncology include both the basic and clinical fields, which is very similar to themes in other departments. Basic research includes both medical physics and radiation biology. Although my majors were clinical and radiation biological research, the lack of personnel was limited to the clinical and medical physics themes at Juntendo. During these years, the department published only a few articles in English; however, this number is now increasing as the number of members in our department has increased. Among these publications, Akamatsu et al. reported a close relationship between the prognosis of patients with esophageal squamous cell carcinoma and the expression of c-erbB-2 in tumor tissue[15]. Kunogi et al. predicted the radiosensitivity of tumor cells by simultaneously detecting histone H2AX phosphorylation and apoptosis[16]. Recently, we reported that patients who underwent radiation therapy for cranial diseases experienced unusual visual and olfactory sensations[17, 18]. Among them, one woman who underwent resection of the olfactory bulb and epithelium reported a pungent smell during the radiation session[19]. This phenomenon suggests that the central nervous system can detect X-rays, even in humans.

Table 2 Changes of the radiation therapy facilities in Juntendo University from 2000 to 2021

	2000	2021
Linear accelerator	2 (H:1, U:1)	7 (H: 3, S:1, U:2, N:1)
Remote after loading system	1 (U:1)	1 (H:1)
Radiation Oncologist	2 (H:2)	11 (H:6, S:2, U:1, N:2)
Medical physicist	0	3 (+2)* (H:2 (+1)*, U:1, N(1)*)

H: Juntendo Hospital, S: Shizuoka Hospital, U: Urayasu Hospital, N: Nerima Hospital,
* numbers in parenthesis mean medical physicists at the faculty of health science

Table 3 Changes of numbers of patients who received radiation therapy at Juntendo Hospital, Hongo, from 2000 to 2020

	2000	2020
Patients who received radiation therapy	450	1003
IMRT	0	271
SRT	32	55
brachytherapy	0	47

IMRT: intensity modulated radiation therapy including VMAT (volumetric-modulated arc therapy)
SRT: stereotactic radiation therapy (including stereotactic radiosurgery)

Conclusions

Radiation therapy has evolved from very primitive techniques to a highly sophisticated and precise level during the past 40 years, along with advances and developments in instrumentation and techniques. It is crucial to stay up to date with these developments to maintain high-quality treatment. The department of radiation oncology is currently small but has gradually expanded year by year. It is my hope that many young investigators will join this field in the future.

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Author contributions

KS contributed to the conception, drafting the manuscript, and preparation of figures and tables.

Conflicts of interest statement

The Author declares that there are no conflicts of interest.

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Original Articles

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The Expression of Rab8, Ezrin, Radixin and Moesin in the Ciliary Body of Cynomolgus Monkeys

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Purpose: The purpose of this study was to determine what proteins are present in the ciliary body (CB). To accomplish this, we conducted a proteomic analysis of the CB of cynomolgus monkeys. We also determined the location of the proteins in CB by immunohistology.

Methods: The eyes of euthanized cynomolgus monkeys were enucleated, and the CB, were isolated from the eyes. Proteins were extracted from the CB and determined by liquid chromatography–mass spectrometry. Separated CB epithelial cells were cultured, and the proteins expressed in the CB were determined by Western blotting. The location of these proteins in the CB was determined by immunohistochemical staining. We also investigated whether adding dexamethasone to the culture medium changed protein expression by the epithelial cells.

Results: Proteomic analysis of the CBs showed that 813 proteins were expressed in the epithelium and stroma. These proteins included the small guanosine triphosphate–binding protein Rab8 and the ezrin/radixin/moesin (ERM) family. Tissue and immunohistological staining confirmed the colocalization of these proteins in non–pigmented CB epithelium. Western blotting of cultured CB epithelial cell lysates showed a tendency that adding dexamethasone changed Rab8 protein expression levels.

Conclusions: Proteomic analysis of CBs identified several proteins involved in the transport and secretion of proteins. These proteins may be involved in the production of aqueous humor and protein secretion by the CB.

Key words: ciliary body, rab8, ezrin, radixin, moesin

Introduction

Glaucoma is a major cause of blindness worldwide: Approximately 60 million people have glaucoma, and approximately 8 million of them are blind in both eyes[1, 2]. The disease is characterized by a progressive loss of retinal ganglion cells, resulting in constriction of the visual fields[2, 3]. Elevated intraocular pressure (IOP) enhances the progression of the disease processes, and a reduction of IOP can slow or block the progression of glaucoma. The IOP can also affect aqueous humor dynamics[4]. Other components that influence IOP are the trabecular meshwork and Schlemm's canal, which are involved in the outflow of aqueous humor, and the ciliary body (CB), which is involved

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in aqueous humor production.

Besides aqueous humor formation, the CB is involved in accommodation and anterior chamber-associated immune deviation. The CB, especially the non-pigmented ciliary epithelium, is also involved in the synthesis and secretion of various proteins found in the aqueous humor that are believed to be involved in controlling IOP[4–6].

Proteins secreted from the CB can affect the trabecular meshwork cells. The glycoproteins in aqueous humor have been shown to be secreted from the CB epithelium[7], and molecules in the aqueous humor, e.g., collagenases, can affect the trabecular meshwork morphology[8,9]. However, a comprehensive description of the proteins present in the aqueous humor has not been published.

Recent advances in proteomics technology have made it possible to perform comprehensive investigations of the proteins in body fluids and tissues. In ophthalmology, proteomics studies have analyzed the proteins in the cells of the trabecular meshwork, aqueous humor, retina, retinal pigment epithelium, and retinal drusen[10–17].

To date, no study has comprehensively determined the proteins expressed in the CB. In addition, the many steps involved in the synthesis and secretion of aqueous humor have not been determined. Therefore, the purpose of this study was to determine the proteins present in the CB. To accomplish this, we conducted a proteomic analysis of the CB of cynomolgus monkeys and thereby focused on the small guanosine triphosphate (GTP)-binding protein Rab8, which has been shown to be involved in vesicle transport and protein localization in small intestinal epithelial cells. Rab8 is also involved in the morphology of the microvilli in small intestinal epithelial[18] and is associated with optineurin[19,20], a glaucoma gene.

Materials and methods

Preparation of cynomolgus monkey eyes

All experimental procedures were approved by the Animal Welfare and Animal Care Committee of the Tsukuba Primate Research Center (TRPC) and the Experimental Animal Committee of the National Tokyo Medical Center. The facilities for housing the monkeys are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Monkeys are routinely examined for physical and ophthalmic conditions by veterinarians and ophthalmologists, and all experiments on monkeys are conducted in accordance with The Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Eyes were obtained in a collaborative study to make effective use of all the monkey tissues for different research programs. Eyes were enucleated immediately after the monkeys were euthanized, and the CBs were isolated from the eyes within 4 hours after death.

Protein extraction from CBs

The tissues from the CBs of a healthy male monkey were homogenized and sonified in lysis buffer (50 mM Tris-HCL, 2 mM EDTA, 0.5% Triton-X, 2% SDS). After centrifugation for 10 minutes at 10 000 rpm (9,300*g*), the supernatant was collected. The protein concentration in the supernatant was determined with the RC DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. Twenty micrograms of the protein sample were combined with an equal volume of 2 × Laemmli buffer and heated for 5 minutes at 100 °C. Then, all samples were stored at -20 °C until use.

Gel digestion and liquid chromatography-mass spectrometry analyses

Twenty micrograms of protein sample was separated on 12.5% acrylamide SDS-PAGE gel. The gel was stained with Colloidal Coomassie Blue (Invitrogen, Carlsbad, CA, USA) and cut into 15 equal pieces of approximately 1 mm^3. The pieces were washed twice with 50mM ammonium bicarbonate/50% acetonitrile, and after destaining, the gel pieces were rinsed with distilled water and incubated with acetonitrile for 20 minutes. The supernatant was discarded, and the gel pieces were completely dried before incubation with 10mM DTT in 100mM ammonium bicarbonate for 45 minutes at 56 °C. The supernatant was discarded, and the pieces were incubated in the dark with 55mM iodoacetamide in 100mM ammonium bicarbonate for 30 minutes at room temperature. Then, the supernatant was discarded, and the gels were washed three times. Finally, the gel pieces were

completely dried before tryptic digestion in sequencing grade trypsin solution (12.5 μg/μL; Thermo Fisher Scientific Inc, Rockford, IL, USA) in 50mM ammonium bicarbonate. The digestion was performed at 37 °C overnight, and then the extraction step was performed once with 25mM ammonium bicarbonate, twice with 5% formic acid, and then once with distilled water. The extracted peptides were pooled and dried. After re-suspending in 40 μL of aqueous 0.01% trifluoroacetic acid/2% acetonitrile, the samples were analyzed by liquid chromatography-mass spectrometry (LCQ Deca XP plus, Thermo Fisher Scientific Inc).

Proteomic analysis of monkey CB

Database searches were performed with the assistance of Bio Works 3.3.1, a protein search program. The UniProt-SwissProt database was initially used by querying the entire theoretical peptide masses provided in the public domain by the Swiss Institute of Bioinformatics. The number of registrations in the UniProt-SwissProt database is about 17 000 for humans and about 14 000 for mice. The data analyses of the monkey CB were aimed at cynomolgus monkeys, humans, orangutans, chimpanzees, rhesus macaques, and lowland gorillas.

Immunohistochemistry of the CB

Enucleated eyes from normal cynomolgus monkeys were fixed in 10% neutralized and buffered formaldehyde solution at 4 °C overnight and then dehydrated. The eyes were embedded in paraffin and serially sectioned at 4 μm thickness. After deparaffinization and rehydration, the specimens were prepared for antigen retrieval by warming in hot water in Target Retrieval Solution (Dako, Glostrup, Denmark) for 30 minutes at 100 °C. The sections were then blocked with phosphate-buffered saline (PBS) containing 10% BSA for one hour and then incubated overnight with primary antibodies (Abs) that were the same as those used for the Western blotting. The slides were washed in PBS and, for nuclear staining, were incubated with Alexa 488 or Alexa 568 (1:500 dilution; Invitrogen) and 4',6'-diamidino-2-phenylindole (DAPI) for one hour at room temperature. The stained tissues were examined with a confocal fluorescence laser microscope (Radiance 2000, Bio-Rad Laboratories). Control slides were prepared by a similar process, but the primary Abs were omitted.

Primary culture of ciliary epithelial cells from cynomolgus monkey

The ciliary epithelial cells isolated from cynomolgus monkey eyes were suspended in DMEM (GIBCO, Carlsbad, CA, USA) containing penicillin-streptomycin (100 U/mL final concentration; Invitrogen) and 10% FBS. Cells were grown to confluence at 37 °C in 5% CO_2. Then, cells were cultured with 100 nM of the glucocorticoid dexamethasone (DEX), 500 nM of timolol malate, or 1 μM of acetazolamide (Sigma-Aldrich, St Louis, MO, USA) for five days.

Protein expression of lysate from blotting of CB

Cultured monkey ciliary epithelial cells were lysed in TNE buffer containing 50mM Tris, 137mM NaCL, 1mM EDTA, 1% TritonX-100, and protease inhibitors (Complete EDTA-free; Roche, Basel, Switzerland). After homogenization and centrifugation for 15 minutes at 14 000 rpm, the supernatant was collected. The protein concentration was determined with the RC-DC protein assay kit (Bio-Rad) according to the manufacturer's instructions. Ten micrograms of proteins from the monkey ciliary epithelium cells lysates were diluted in an equal volume of 2×Laemmli buffer and heated for 5 minutes at 100 °C. Samples were separated by 7.5% SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membrane. Membranes were blocked in PBS containing 0.05% tween20 (PBS-T) and 5% non-fat dry milk for one hour and probed overnight at 4 °C with one of the following primary Abs: rabbit anti-Rab8 Ab (Sigma-Aldrich), goat anti-Ezrin Ab (Sigma-Aldrich), goat anti-Radixin Ab (Sigma-Aldrich), goat anti-Moesin Ab (Sigma), and mouse anti-Actin Ab (Millipore, Billerica, MA, USA). The specific signals were detected with horseradish peroxidase (HRP)-conjugated donkey Ab to goat IgG or rabbit IgG as secondary Abs. The signals were made visible by chemiluminescence reactions and examined with a ChemiDoc XRS plus (Bio-Rad).

Results

Proteomic analysis of monkey CB

The proteomic analysis of proteins extracted from the stroma and epithelium of the monkey CB

identified a total of 813 proteins. Detailed information on these proteins is shown as supplemental data. These proteins were classified by a biological process registered in the Gene Ontology database that uses pathway tool software (MetaCore, Gene GO, Infocom; Figure 1). The proteins identified in the CB are involved in protein transport, localization, and secretion (Table 1).

Immunohistochemical analyses

The results of proteomic analysis of monkey CB included the Rab8 protein (No.170), which is a small GTP-binding protein that is related to the glaucoma gene optineurin, and is known to be involved in protein localization and transportation in the small intestine and can affect the morphology of microvilli[18]. The ezrin (No.70)/radixin (No.160)/moesin (No.108), ERM family, which are core proteins in the microvilli that also act as linker

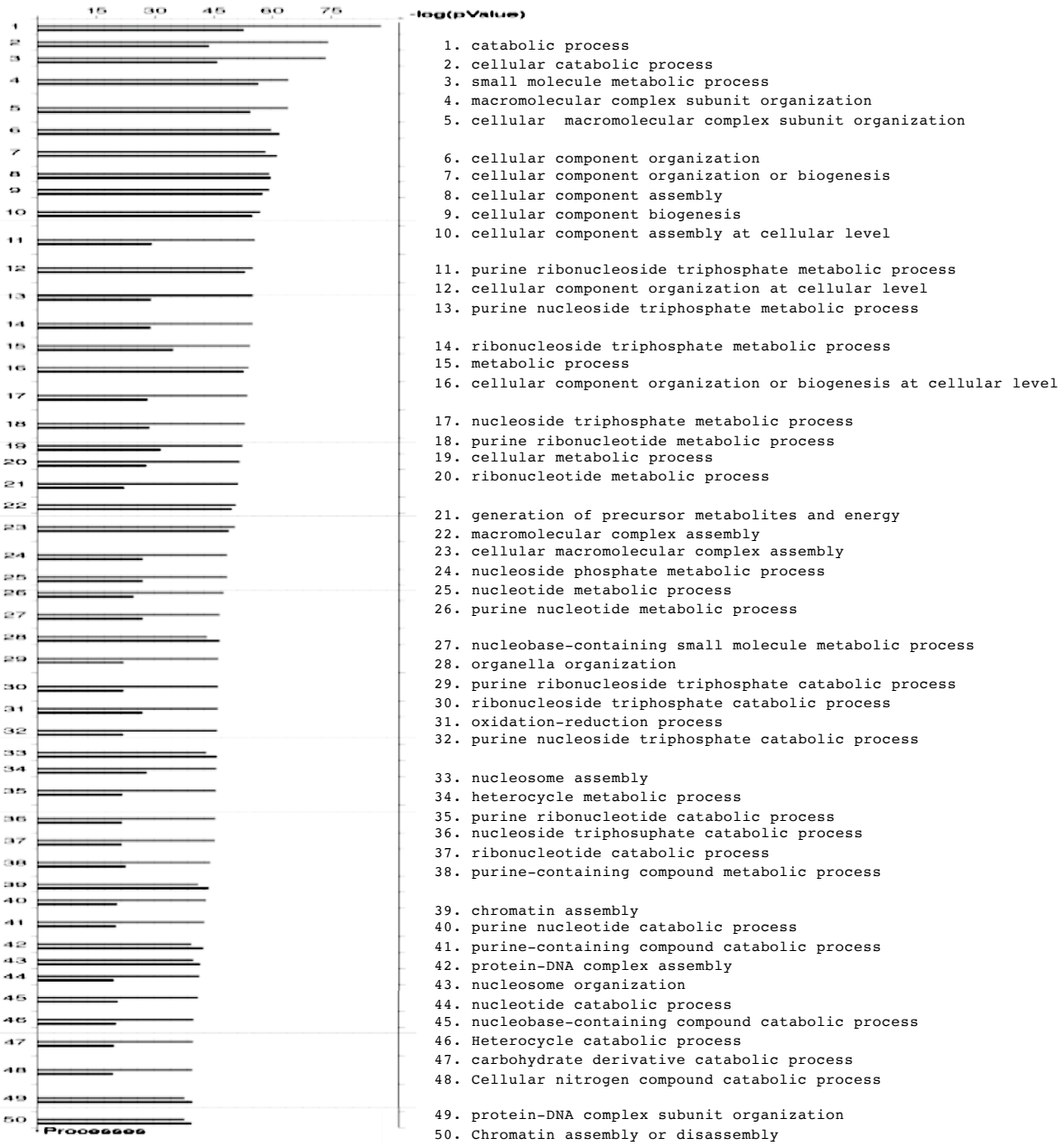

Figure 1 Classification according to the Gene Ontology database

The pathway tool software (MetaCore,Gene GO,Infocom) was used to classify all of the identified proteins by the biological process registered in the Gene Ontology database.

Upper bar, proteins identified mainly in the ciliary epithelium; lower bar, proteins identified mainly in the ciliary stroma

Table 1 Principal proteins of cynomolgus monkey ciliary body

No.	Protein name	Database accession No.	MW (Da)	Sequence coverage (%)	No. of peptide
1	10 kDa heat shock protein, mitochondrial	P61604	10924.9	25.50	3
2	14 kDa phosphohistidine phosphatase	Q9NRX4	13823.7	16.80	1
3	14-3-3 protein beta/alpha	P31946	28064.8	10.20	5
4	40S ribosomal protein S10	P46783	18885.9	5.50	2
5	60 kDa heat shock protein, mitochondrial	P10809	61016.5	17.60	10
6	60S acidic ribosomal protein P0	P05388	34251.8	3.50	2
7	78 kDa glucose-regulated protein	P11021	72288.5	13.80	7
8	Acetylcholinesterase	P22303	67753.4	2.10	1
9	Aconitate hydratase, mitochondrial	Q99798	85372.0	19.90	31
10	Actin, alpha cardiac muscle 1	P68032	41991.9	46.40	181
11	Acyl-protein thioesterase 1	O75608	24653.5	6.10	1
12	ADAM 21	Q9UKJ8	80766.1	1.10	1
13	Adenomatous polyposis coli protein	P25054	311453.1	0.50	1
14	Adenosylhomocysteinase	P23526	47685.3	3.50	1
15	ADP/ATP translocase 1	P12235	33043.2	17.80	28
16	Aldose 1-epimerase	Q96C23	37742.1	4.10	1
17	Alpha-2-macroglobulin	P01023	163174.3	7.60	16
18	Alpha-actinin-1	P12814	102992.7	20.50	30
19	Alpha-adducin	P35611	80904.8	2.40	1
20	Alpha-crystallin A chain	P02489	19896.9	31.20	5
21	Alpha-enolase	P06733	47139.4	24.40	34
22	Alpha-internexin	Q16352	55357.5	2.40	8
23	Annexin A1	P04083	38690.0	20.20	6
24	Apolipoprotein O	Q9BUR5	22270.5	23.20	3
25	Arachidonate 5-lipoxygenase	P09917	77933.4	2.10	2
26	Aspartate aminotransferase, cytoplasmic	P17174	46218.6	11.10	4
27	ATP synthase subunit alpha, mitochondrial	P25705	59713.7	21.00	32
28	Beta-actin-like protein 2	Q562R1	41976.0	12.00	92
29	Beta-crystallin A3	P05813	25133.8	7.00	1
30	Beta-enolase	P13929	46957.4	11.10	11
31	Calcium-binding mitochondrial carrier protein Aralar1	O75746	74709.0	4.90	4
32	Calnexin	P27824	67526.0	9.10	3
33	Calponin-1	P51911	33149.6	10.40	4
34	Calreticulin	P27797	48111.9	4.30	2
35	Carbonic anhydrase 2	P00918	29227.9	3.50	1
36	Caskin-2	Q8WXE0	126633.8	0.00	1
37	Catenin alpha-1	P35221	100008.6	2.00	1
38	Cationic amino acid transporter 3	Q8WY07	67125.6	1.90	2
39	CD59 glycoprotein	P13987	14167.8	8.60	4
40	CD9 antigen	P21926	25399.0	5.30	2
41	Centromere protein C 1	Q03188	106860.4	1.30	1
42	Citrate synthase, mitochondrial	O75390	51679.6	7.70	4

43 Clathrin heavy chain 1	Q00610	191491.7	6.30	12
44 Coatomer subunit alpha	P53621	138243.8	1.00	1
45 Cofilin-1	P23528	18490.7	28.30	9
46 Complement C3	P01024	187029.3	1.00	1
47 Contactin-2	Q02246	113322.6	1.40	1
48 Creatine kinase B-type	P12277	42617.4	32.50	21
49 Cystatin-B	P04080	11132.6	12.20	1
50 Cytochrome c	P99999	11741.1	33.30	13
51 Cytoplasmic aconitate hydratase	P21399	98336.7	1.60	1
52 Cytoskeleton-associated protein 5	Q14008	225364.6	0.80	1
53 Dachshund homolog 1	Q9UI36	78513.4	2.00	1
54 D-dopachrome decarboxylase	P30046	12703.7	9.30	2
55 Decorin	P07585	39721.9	4.20	1
56 Density-regulated protein	O43583	22078.1	6.60	1
57 Desmin	P17661	53503.2	39.40	65
58 Destrin	P60981	18493.5	28.50	10
59 Diacylglycerol kinase epsilon	P52429	63885.0	0.00	1
60 Dihydropteridine reductase	P09417	25773.0	12.30	2
61 Dihydropyrimidinase-related protein 1	Q14194	62144.8	2.80	1
62 DmX-like protein 2	Q8TDJ6	339541.7	0.50	1
63 DNA mismatch repair protein Msh2	P43246	104676.8	0.00	1
64 DNA polymerase kappa	Q9UBT6	98745.8	1.70	1
65 Dynamin-2	P50570	98003.3	1.60	2
66 Dynein heavy chain 5, axonemal	Q8TE73	528683.8	0.30	1
67 Dysferlin	O75923	237142.3	0.00	1
68 Dystrophin	P11532	426426.0	0.40	2
69 Electron transfer flavoprotein subunit alpha, mitochondrial	P13804	35057.6	8.70	2
70 Elongation factor 1-alpha 1	P68104	50109.2	7.10	6
71 Endoplasmin	P14625	92411.2	2.40	2
72 Endothelin-converting enzyme-like 1	O95672	87735.7	3.40	2
73 Eyes absent homolog 3	Q99504	62519.0	3.10	1
74 Ezrin	P15311	69369.8	3.20	2
75 Ferritin heavy chain	P02794	21212.3	13.10	6
76 Fibrillin-1	P35555	312097.0	0.50	1
77 Filamin-A	P21333	280561.4	2.80	11
78 Fructose-bisphosphate aldolase A	P04075	39395.3	9.30	5
79 Fumarate hydratase, mitochondrial	P07954	54602.2	7.30	2
80 Fumarylacetoacetate hydrolase domain-containing protein 2A	Q96GK7	34574.1	16.60	5
81 Galectin-1	P09382	14706.2	32.60	5
82 Gamma-enolase	P09104	47239.1	7.60	5
83 Gelsolin	P06396	85644.3	14.10	16
84 Glucose-6-phosphate isomerase	P06744	63107.3	11.50	5
85 Glutamate receptor 2	P42262	98758.3	1.80	3
86 Glutathione S-transferase kappa 1	Q9Y2Q3	25480.3	10.60	3
87 Glyceraldehyde-3-phosphate dehydrogenase	P04406	36030.4	40.00	63

88 Haptoglobin	P00738	45176.6	13.30	7
89 Heat shock 70 kDa protein 1	P08107	70009.2	9.00	9
90 Hemoglobin subunit alpha	P69905	15247.9	23.90	10
91 Hexokinase-1	P19367	102420.2	3.20	3
92 Histone H4	P62805	11360.4	53.40	22
93 Ig alpha-1 chain C region	P01876	37630.7	15.60	4
94 Inactive phospholipase C-like protein 2	Q9UPR0	125785.3	1.00	6
95 Interphotoreceptor retinoid-binding protein	P10745	135277.8	1.10	1
96 Kallikrein-12	Q9UKR0	26716.2	6.50	1
97 Kappa-actin	Q9BYX7	41988.9	4.30	64
98 Kelch-like protein 8	Q9P2G9	68757.9	1.60	1
99 Kinesin-like protein KIF23	Q02241	109989.7	0.70	1
100 Lactoylglutathione lyase	Q04760	20764.3	4.30	1
101 Lamin-A/C	P02545	74094.8	21.80	30
102 Laminin subunit alpha-4	Q16363	202399.2	0.90	2
103 L-lactate dehydrogenase A chain	P00338	36665.4	3.60	2
104 Lutheran blood group glycoprotein	P50895	67362.7	4.80	8
105 Macrophage migration inhibitory factor	P14174	12468.2	7.80	2
106 Malate dehydrogenase, cytoplasmic	P40925	36403.0	23.10	18
107 Microsomal glutathione S-transferase 3	O14880	16505.6	17.80	3
108 Moesin	P26038	67777.9	5.50	2
109 Mu-crystallin homolog	Q14894	33754.4	7.30	2
110 Myelin basic protein	P02686	33097.3	11.20	7
111 Myosin-1	P12882	222975.5	0.50	3
112 Myotrophin	P58546	12886.6	30.50	2
113 Nesprin-1	Q8NF91	1010433.0	0.10	1
114 Nestin	P48681	177331.0	1.10	1
115 Neuroblastoma-amplified gene protein	A2RRP1	268412.5	0.00	1
116 Neurofilament heavy polypeptide	P12036	112412.0	0.90	8
117 Neurolysin, mitochondrial	Q9BYT8	80599.8	0.00	2
118 Neutral alpha-glucosidase AB	Q14697	106806.8	2.40	1
119 Nucleolar protein 14	P78316	97607.5	1.50	1
120 Nucleoside diphosphate kinase 3	Q13232	19002.9	10.10	2
121 Ovochymase-1	Q7RTY7	124985.6	1.10	1
122 P2X purinoceptor 1	P51575	44951.2	3.80	1
123 Palmitoyl-protein thioesterase 1	P50897	34171.3	7.80	1
124 Peptidyl-prolyl cis-trans isomerase A	P62937	18000.9	64.20	29
125 Periaxin	Q9BXM0	154906.0	4.90	2
126 Peripherin	P41219	53618.5	6.60	16
127 Peroxiredoxin-1	Q06830	22096.3	24.60	6
128 Phosphate carrier protein, mitochondrial	Q00325	40068.8	9.90	19
129 Phosphatidylethanolamine-binding protein 1	P30086	21043.7	27.30	11
130 Phosphoglucomutase-1	P36871	61410.6	5.70	3
131 Phosphoglycerate mutase 1	P18669	28785.9	31.50	12
132 Plasma membrane calcium-transporting ATPase 3	Q16720	134112.2	1.50	1

133 Plasminogen	P00747	90510.2	2.10	1
134 Plectin-1	Q15149	531407.9	0.30	1
135 Potassium channel subfamily K member 3	O14649	43490.0	0.00	1
136 Pre-B-cell leukemia transcription factor-interacting protein 1	Q96AQ6	80594.2	4.20	3
137 Pregnancy zone protein	P20742	163728.1	1.50	4
138 Prenylcysteine oxidase 1	Q9UHG3	56603.8	3.20	1
139 Proactivator polypeptide	P07602	58073.9	2.10	1
140 Probable phosphoglycerate mutase 4	Q8N0Y7	28758.8	11.80	7
141 Profilin-2	P35080	15036.3	10.00	2
142 Prohibitin-2	Q99623	33275.9	14.00	4
143 Prolargin	P51888	43782.2	2.90	1
144 Prolyl endopeptidase	P48147	80712.1	1.80	1
145 Prostaglandin E synthase 3	Q15185	18685.4	6.30	1
146 Proteasome subunit beta type-3	P49720	22933.5	7.80	1
147 Protein AF-10	P55197	108958.5	1.10	1
148 Protein disulfide-isomerase	P07237	57080.8	3.10	3
149 Protein DJ-1	Q99497	19878.5	7.90	3
150 Protocadherin-15	Q96QU1	215932.5	0.80	1
151 Putative annexin A2-like protein	A6NMY6	38634.8	11.20	14
152 Putative elongation factor 1-alpha-like 3	Q5VTE0	50153.2	4.80	6
153 Putative GTP-binding protein RAY-like	Q9BW83	20467.3	4.30	1
154 Putative heat shock 70 kDa protein 7	P48741	40219.6	3.50	2
155 Putative histone H2B type 2-C	Q6DN03	21458.2	5.20	7
156 Putative nucleoside diphosphate kinase	O60361	15519.0	33.60	6
157 Putative RNA methyltransferase NOL1	P46087	89247.2	1.40	1
158 Putative tubulin beta-4q chain	Q99867	48403.5	5.80	8
159 Pyruvate kinase isozymes M1/M2	P14618	57900.2	35.20	32
160 Radixin	P35241	68521.5	5.80	3
161 Ras suppressor protein 1	Q15404	31520.7	6.10	1
162 Ras-related C3 botulinum toxin substrate 1	P63000	21436.3	5.20	1
163 Ras-related protein Rab-10	P61026	22526.6	6.00	1
164 Ras-related protein Rab-11A	P62491	24378.4	14.40	4
165 Ras-related protein Rab-1A	P62820	22663.4	16.10	2
166 Ras-related protein Rab-2A	P61019	23530.8	6.60	1
167 Ras-related protein Rab-3A	P20336	24968.1	7.30	1
168 Ras-related protein Rab-5C	P51148	23467.8	6.50	1
169 Ras-related protein Rab-7a	P51149	23474.9	6.80	1
170 Ras-related protein Rab-8A	P61006	23653.2	6.80	1
171 Ras-related protein Rap-1A	P62834	20973.7	13.60	3
172 Ras-related protein Rap-1b	P61224	20811.6	13.60	3
173 Retinal dehydrogenase 1	P00352	54827.0	3.00	1
174 Rho GDP-dissociation inhibitor 1	P52565	23192.7	7.40	6
175 RNA-binding protein 44	Q6ZP01	117910.9	1.30	2
176 Secernin-1	Q12765	46352.6	3.10	1
177 Selenium-binding protein 1	Q13228	52357.7	16.10	4

178 Septin-2	Q15019	41461.3	8.00	2
179 Serotransferrin	P02787	76999.7	17.80	12
180 Serum albumin	P02768	69321.6	22.50	74
181 Sideroflexin-3	Q9BWM7	35480.5	4.70	1
182 Signal peptide peptidase-like 2C	Q8IUH8	74404.0	2.20	2
183 Sodium/potassium-transporting ATPase subunit alpha-1	P05023	112824.1	8.70	15
184 Solute carrier family 2, facilitated glucose transporter member 1	P11166	54048.7	2.00	3
185 Sorcin	P30626	21662.4	5.60	1
186 Spectrin alpha chain, brain	Q13813	284362.5	7.00	18
187 Stomatin-like protein 2	Q9UJZ1	38510.2	4.20	2
188 Stress-70 protein, mitochondrial	P38646	73634.8	16.60	10
189 Superoxide dismutase [Mn], mitochondrial	P04179	24706.6	16.20	3
190 Talin-1	Q9Y490	269596.3	1.60	5
191 Tektin-4	Q8WW24	50617.3	0.00	1
192 Thioredoxin-dependent peroxide reductase, mitochondrial	P30048	27675.2	12.10	4
193 Titin	Q8WZ42	3812906.0	0.00	1
194 Transgelin	Q01995	22596.4	16.40	6
195 Transketolase	P29401	67834.9	6.40	8
196 Translationally-controlled tumor protein	P13693	19582.6	15.10	2
197 Trichoplein keratin filament-binding protein	Q9BT92	61034.3	2.40	2
198 Trifunctional enzyme subunit alpha, mitochondrial	P40939	82947.0	8.50	8
199 Triosephosphate isomerase	P60174	26652.7	57.00	26
200 Tropomyosin alpha-1 chain	P09493	32688.7	27.50	22
201 Tubulin alpha-1A chain	Q71U36	50103.7	16.20	25
202 Tyrosine aminotransferase	P17735	50366.5	3.10	1
203 Ubiquitin	P62988	8559.6	32.90	8
204 Vacuolar proton pump subunit E 1	P36543	26128.8	6.20	1
205 Vesicle-associated membrane protein 2	P63027	12640.7	20.70	6
206 Vimentin	P08670	53619.2	52.10	134
207 Vinculin	P18206	123721.9	3.50	4
208 Voltage-dependent anion-selective channel protein 1	P21796	30753.6	23.00	6
209 Wolframin	O76024	100241.2	1.50	1
210 Zinc finger protein 577	Q9BSK1	54122.0	3.30	1

proteins between actin filaments and the cell membranes. Rab8 and moesin have also been reported to interact in the photoreceptor cells[21]. It is reasonable to assume that Rab8 and ERM family interact also in CB, and are involved in protein secretion. Double immunostaining with anti-Rab8 Ab and anti-ERM family Abs showed expression of Rab8, ezrin, radixin, and moesin in the CB epithelium and especially in the non-pigmented epithelium. A co-expression of Rab8 and the ERM family proteins was also detected in the ciliary epithelium (Figure 2A, 2B, 2C) and primary cultured ciliary epithelial cells (Figure 2D).

Drug effects on ciliary epithelium cells in vitro

The secondary glaucoma caused by glucocorticoids is both clinically and morphologically similar to primary open angle glaucoma. When trabecular meshwork cells are exposed to DEX, the extracellular matrix increases and the trabecular meshwork thickens, and resulting in IOP elevation. It is known that glucocorticoids affect IOP. However,

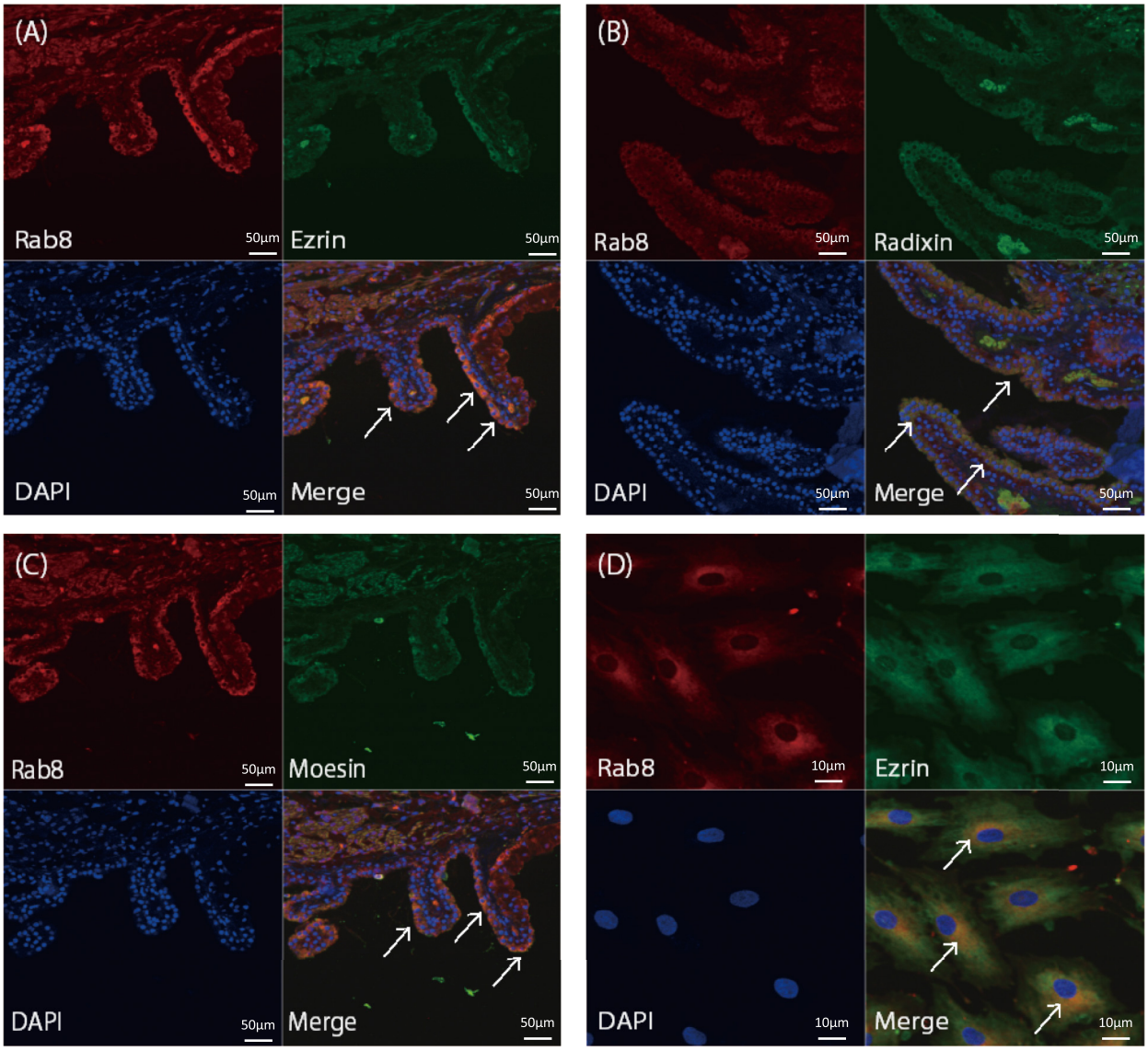

Figure 2 Immunohistochemistry of normal cynomolgus monkey ciliary body, and immunofluorescence staining of cultured monkey ciliary body cells

Paraffin sections of cynomolgus monkey ciliary body (CB) were labeled with antibodies specific to Rab8 (A–C), ezrin (A), radixin (B), and moesin (C). Monkey CB cells were stained with Rab8 and ezrin (D). Co-expression of Rab8 and the ezrin/radixin/moesin (ERM) family can be seen at the ciliary non-pigmented epithelium cells (arrow).

the relationship between CB function and glucocorticoids remains unclear. We investigated whether Rab8 interacts the mechanism of IOP elevation by glucocorticoids stimulation. Light microscopy did not show any morphological changes in the morphology and protein expression of cultured cynomolgus monkey non-pigmented ciliary epithelial cells with different concentrations of DEX (data not shown). Western blotting of epithelial cell lysates showed a tendency for the addition of DEX to decrease the expression of Rab8 protein and this effect seemed dose-dependent, though this is not significant (Figure 3A). The experiments repeated twice and the results were similar. In addition, when cells were cultured in the same way and timolol maleate or acetazolamide was added, no changes were observed in the expression of Rab8 (–Figure 3B, 3C).

Discussion

Proteomic analyses of the CB of cynomolgus monkeys detected many proteins, some of which are involved in protein transport and secretion. In the CB, the difference of protein characteristics was found between ciliary epithelium and stroma. Structure proteins were major component in stroma,

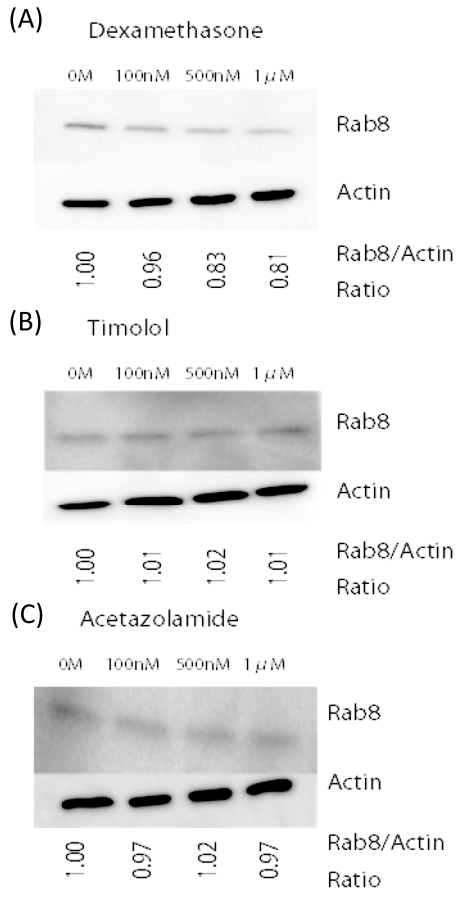

Figure 3 Effect of dexamethasone on the expression of Rab8 in monkey ciliary epithelial cells

A: Western blot analysis with an antibody against Rab8 showed a tendency of decrease in the expression of Rab8 in monkey ciliary epithelial cells after exposure to dexamethasone (Dex) for 5 days. Western blot analysis with an antibody against actin was the control. The relative amount of Rab8/actin was quantified.

B: Western blot analysis of ciliary epithelial cells after exposure to timolol maleate for five days. No change was seen in the expression of Rab8 proteins.

C: Western blot analysis of ciliary epithelial cells after exposure to acetazolamide for five days. No change was seen in the expression of Rab8 proteins.

and catalytic and metabolic proteins were more expressed in epithelium than stroma. Diffusion, ultrafiltration, and active secretion are physiological processes that participate in the production of aqueous humor, especially active secretion[4]. Blood flows through the CB, and aqueous humor is produced from the blood plasma by the non-pigmented ciliary epithelium. The proteins in the aqueous humor differ both quantitatively and qualitatively from those in the plasma[4, 22, 23], indicating that the CB must be involved in the secretion of proteins into the aqueous humor. In comparison of CB with aqueous humor, Chowdhury et al reported that 355 proteins were identified from human aqueous humor by narrow liquid chromatography electrospray ionization tandem mass spectrometry, and most of the proteins had catalytic, enzymatic, and structural properties[23]. Catalytic and metabolic proteins are almost 30% each of top 50 distribution by function by Gene Ontology in CB, and approximately 20% each in aqueous humor. Structure proteins take up around 35% in CB, whereas 9.9% in aqueous humor[23], though simple comparison is not possible according to different way of classification.

Myocilin, a glaucoma gene, is found in the aqueous humor, and the trabecular meshwork cells are known to secrete exosomes into the aqueous humor[24]. The CB appears to secrete proteins into the aqueous humor in the same way.

Rab8, one of the proteins identified by our proteomic analysis, is a small GTP-binding protein that is related to optineurin, a glaucoma gene[18–20]. It is known to be involved in protein localization and transportation in the small intestine and can affect the morphology of microvilli[18]. Although no reports have described the role that Rab8 plays in the CB, it is quite reasonable to assume that it is involved in protein secretion.

Our proteomic analysis also identified the ERM family proteins, which are core proteins of the microvilli and are found directly beneath the cell membranes. They act as linker proteins and connect actin filaments to the cell membranes[25, 26]. Rab8 and moesin have been reported to interact to regulate the transport of rhodopsin in photoreceptor cells[21]. Both proteins appear to be related to protein transport and to be active in the CB.

We double immunostained for Rab8, ezrin, radixin, and moesin in cynomolgus monkey CB tissues and also in cultured non-pigmented ciliary epithelial cells. Confocal microscopy showed a co-expression of Rab8 and the ERM family in the non-pigmented epithelium of the CB, suggesting that these proteins are involved in the function of the non-pigmented epithelium of the CB.

The secondary glaucoma caused by glucocorticoids is both clinically and morphologically similar to primary open angle glaucoma. Thus, glucocorticoids have been used in both *in vivo* and *in vitro* glaucoma research[27–31]. When trabecular mesh-

work cells are exposed to DEX, the extracellular matrix increases and the trabecular meshwork thickens, resulting in an elevation of IOP[31]. It is also widely known that glucocorticoids affect IOP. However, the relationship between CB function and glucocorticoids remains unclear.

We cultured non-pigmented ciliary epithelial cells and found a tendency for adding DEX to reduce the level of Rab8 expression in these cells. Although drugs such as beta-blockers and acetazolamide can alter the production of aqueous humor, we did not observe any changes in the level of Rab8 expression when timolol maleate or acetazolamide was added to cultured CB epithelial cells. We suggest that these proteins influence the function and morphology of the trabecular meshwork. They may also affect the aqueous humor outflow route and thus the IOP. Determining these functions would be relevant for understanding the mechanism of IOP control and glaucoma pathogenesis.

Although Rab8 might be involved in functions in the CB besides the secretion of aqueous humor, the finding that Rab8 expression in the CB was affected by DEX stimulation indicates that Rab8 may have some effect on IOP dynamics. To obtain more information on this relationship, analyses of the CB epithelial cell secretome and secreted exosomes and experiments involving co-culture models with trabecular meshwork cells are required.

In conclusion, Rab8 probably plays some role in the secretion of aqueous humor in the CB of cynomolgus monkeys and co-exists with ERM family molecules. Its expression is dependent on stimulation by glucocorticoids. Further studies will be needed to investigate the exact role of Rab8 in the CB.

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Author contributions

KT and IK collected and analyzed the experimental date, drafted the manuscript. HO, AC, MA and NS supported the experiment, NE, AM and TI conceived and participated in the study design and critically reviewed the manuscript.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Original Articles

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Action and Contribution of the Iliopsoas and Rectus Femoris as Hip Flexor Agonists Examined with Anatomical Analysis

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Objectives: To evaluate the difference in action between the iliopsoas and rectus femoris muscles in hip flexion by estimating the relative contribution to the maximal hip flexion torque and relative rotation speed.
Materials: We examined 22 lower limbs of 10 male and 12 female formaldehyde-fixed adult Japanese cadavers.
Methods: Using morphometric data from cadaver dissections, we calculated the moment arm length and physiological cross-sectional area for each muscle. We considered moment arm length and physiological cross-sectional area as indices of the maximal torque and compared them among the muscles at various hip joint angles. To evaluate the relative rotation speed, we calculated the increase of the hip joint angle for a 1% reduction of the muscle fiber length in each muscle.
Results: The rectus femoris contributed approximately 2/3 to the flexion torque in mild flexion up to 60°, whereas the iliopsoas contribution increased sharply beyond 60°. The relative iliopsoas rotation speed was 2.5- to 3-times higher than that of the rectus femoris in mild flexion up to 60° under the specific condition that each muscle had the same muscle contraction speed.
Conclusions: We found that the iliopsoas served as a rapid flexor, while the rectus femoris was a powerful flexor.
Key words: hip joint, iliopsoas, rectus femoris, physiological cross-sectional area, torque

Introduction

Among the activities of daily living, standing up, as well as walking, constitute the most critical and fundamental exercises[1–2)]. In these exercises, flexion and extension of the hip and knee joints are essential and critical motor elements[3)]. The extension of the hip and knee joints plays an important role during chair-based standing and sitting. Various studies have analyzed the biomechanics of these extension activities using force plates[1, 4)], motion analysis[2, 4, 5)], and electromyography (EMG)[1, 2, 4, 6)]. The walking and running exercises involve flexion of the hip and knee joints. Based on their observa-

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tions of larger hip flexor muscles in top sprinter athletes, Ema et al[7]. reported that hip flexion could play an important role in track and field sprinting. Biomechanical analysis results of these flexion activities have been reported in studies employing EMG of the iliopsoas and rectus femoris[8] or combining EMG and hip flexion torque measurement[9].

Joint movements are exerted in general by multiple muscles. During knee extension, the four muscles of the quadriceps femoris play the role of the main agonist. During hip extension, the gluteus maximus and three muscles of the hamstrings play the role of main agonists, and the adductor maximus acts as a synergist. During hip flexion, the iliopsoas and rectus femoris are the main agonists, and the sartorius and adductor longus act as synergists. Dynamometers measure the total torque produced by multiple agonists and synergists. However, it cannot discriminate the muscle strength of individual muscles. The EMG estimates the change of activities in individual muscles during joint movements. However, it cannot predict the relative muscle strength among the agonists and synergists. The EMG activities of the superficially located rectus femoris have been reported in many studies[7, 10–17], and those of the deeply located iliopsoas have also been reported in some studies[8, 18–23].

The morphometric parameters of the skeletal muscles, such as the muscle fiber length (FL) and physiological cross-sectional area (PCSA), are well known to affect the muscle function, including the contraction speed, maximum muscle strength, and the effective contractible range[24, 25]. Indeed, the agonists and synergists of the hip and knee joints reportedly have different architectural parameters and functional characteristics[26], suggesting that the multiple flexors and extensors of the hip and knee joints have varying contributions to the torque and speed during joint movement. However, the contribution of the individual muscles was not hitherto revealed by the previous studies employing dynamometers, EMG, and other physiological methods.

The iliopsoas (psoas major and iliacus), rectus femoris, sartorius, and adductor longus are known as the hip flexor muscles. The iliopsoas and rectus femoris are considered agonist muscles among the hip flexor muscles as they have greater PCSA values[26]. In comparison, the sartorius and adductor longus have much smaller PCSA values, and their force vector is deviated from the sagittal plane, so they are thought to have only minor contributions compared with the iliopsoas and rectus femoris. In the present study, we determined the relative contribution of the iliopsoas and rectus femoris to the maximal hip flexion torque based on morphological methods by estimating PCSA and moment arm length (MAL) values in the sagittal plane. Additionally, based on the morphometric data of the skeletal anatomical specimens, we estimated the flexion speed of individual muscles by calculating the degree of flexion produced by 1% of shortening of the hip flexor muscles. The data provided by the present methods were not relevant to estimate the actual torque and speed of the joint movements but revealed the maximal and relative contributions of the individual muscles on a theoretical basis.

Materials and Methods

Materials

We used 22 lower limbs of 10 male and 12 female formaldehyde-fixed adult Japanese cadavers with no apparent degeneration in the hip and knee joints that were used in the dissection course at the Nihon University School of Dentistry during the 2014 and 2016 academic years. For morphometry and estimation of the PCSA of the iliopsoas and rectus femoris, 12 specimens from 6 male and 6 female cadavers (age at death, 78.6 ± 10.6 years) were used, and for measurement of the MAL, 10 specimens from 4 male and 6 female cadavers (age at death, 79.6 ± 6.8 years) were used. The body donors gave written informed consent for the donation of their tissues for research and teaching purposes. The study was carried out following all relevant guidelines and regulations and was approved by the ethical committee of the Nihon University School of Dentistry (EP14D009, EP16D017).

Procedures/protocol

Morphometry and calculation of PCSA in isolated muscle specimens

In the 12 lower limbs, the iliopsoas and rectus femoris were dissected out to prepare the isolated muscle specimens as stated previously[27, 28].

In the isolated muscle specimens, the mass (M), FL, and pennation angle (θ) were measured. The

pennation angles were measured with a protractor at the distal myotendinous junction as the angular deviation between the muscle fibers and tendon[28]. The FL and pennation angles were measured and averaged in three places (superficial, intermediate, and deep portions) on the iliopsoas and in three places (on the left, right, and distal sides of the origin tendon) on the rectus femoris.

Based on these data, the PCSA was calculated using the following formula, with the density of mammalian muscles represented by ρ (ρ=1.056 g/cm^3)[29]:

PCSA (cm^2) = M (g) × cos θ/ρ (g/cm^3) × FL (cm)[30-32].

Measurement of muscle MAL in skeletal specimens

The 10 lower limbs were dissected, and the locations of the origin and insertion of the iliopsoas (the psoas major and iliacus) and rectus femoris were marked on the skeleton. Then, the muscles were removed to prepare the skeleton specimens. The diameter of the femoral head was measured after opening the hip joint and removing the iliofemoral and pubofemoral ligaments covering the head. Three-dimensional reference values were obtained with two reflective markers for the origin and insertion of the muscle on the skeleton, and four reflective markers on the surface of the femoral head for the joint center of the hip joint.

A) Measurement of three-dimensional reference values in the hip extension position.

The MAL in the hip extension position was calculated from the three-dimensional reference values of the reflective markers attached to the skeleton that were measured with four infrared cameras and an optical motion capture system (NaturalPoint, Inc., Corvallis, OR, USA). The origin of the psoas major was marked on the T12 for the superficial head and on the costal process of L1 for the deep head, and the insertion was marked on the lesser trochanter of the femur[33]. The origin and insertion of the iliacus were marked on top of the iliac crest and the lesser trochanter, respectively. The origin and insertion of the rectus femoris were marked on the ridge of the anterior inferior iliac spine and the tibial tuberosity, respectively. The center of the hip joint was determined from the four markers attached to the femoral head surface, and the offset distance in the direction of the ball center representing the radius was determined by direct measurement.

B) Estimation of MAL under different hip joint angles in the sagittal plane.

The MAL of the muscles was calculated from the three-dimensional reference values of the reflection markers projected on the sagittal plane as the distance between the center of the hip joint and the vector of the muscle force by Skycom software (Optitrack Japan, Tokyo, Japan). The iliopsoas did not take a straight course between the origin and the insertion in the hip extension positions in various degrees up to 60°, but a curved course around the femoral head, so that the MAL was represented by the radius of the femoral head.

The psoas major (PM) had two heads whose origins were represented by the T12 vertebral body for the superficial head (PM-I) and by the L1 transverse process for the deep head (PM-II). The iliacus (IL) had a broad origin region on the iliac fossa, and the origins were represented by four points on the iliac crest including the anterior superior iliac spine (IL-I), the midpoint between the former and the apex of the iliac crest (IL-II), the apex of the iliac crest (IL-III), and the posterior superior iliac spine (IL-IV). The MAL was represented by the radius of the femoral head for IL-I in joint angles up to 10°, IL-II up to 30°, PM-I up to 50°, and IL-III, IL-IV, and PM-II up to 60°, and by the length of the vertical line from the hip joint center to the vector from the origins to the insertion (lesser trochanter of the femur) in larger joint angles. We employed the mean value of the MAL of PM-I and PM-II for the PM, and of IL-I to IL-IV for the IL.

For the rectus femoris, the MAL was represented by the length of the vertical line from the hip joint center to the vector from the origin (inferior anterior iliac spine) to the insertion (tibial tuberosity) in all of the angle regions (Figure 1).

Estimation of the relative contribution to the maximal hip flexion torque

To estimate the relative contribution of the individual flexors to the maximal hip flexion torque, we calculated an indicator of maximal hip flexion

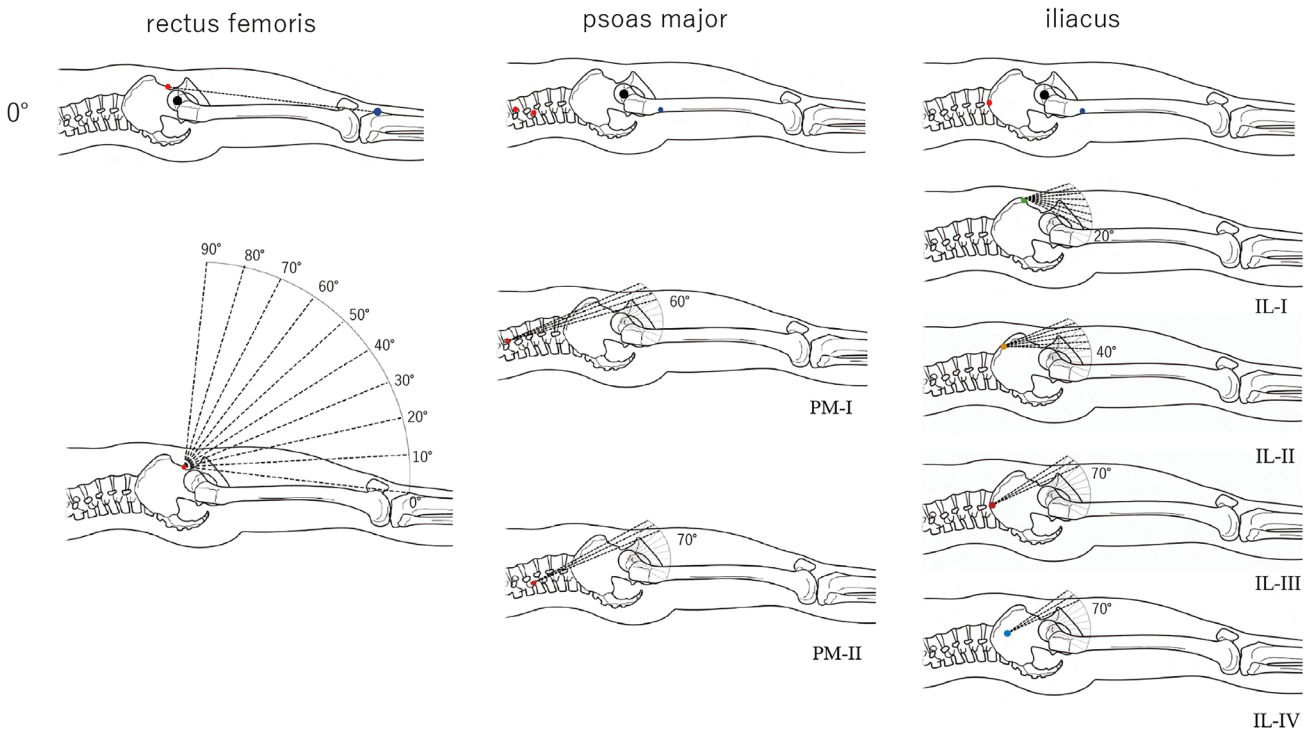

Figure 1 Measurement of moment arm length (MAL) of the three muscles under different hip joint angles.
For the rectus femoris, the MAL was represented by the length of the vertical line from the hip joint center (black dot) to the vector from the origin (red dot) to the insertion (blue dot) in all angle regions. For the psoas major (PM), PM had two heads, and the origins were represented by the T12 vertebral body for the superficial head (PM-I) and by the L1 transverse process for the deep head (PM-II). The MAL was represented by the radius of the femoral head for PM-I up to 50° and for PM-II up to 60°, and by the length of the vertical line from the hip joint center to the vector from the origins (red dot) to the insertion in larger joint angles. The iliacus (IL) had a broad origin region on the iliac fossa, which were represented by 4 points (green dot, yellow dot, red dot, and light blue dot) on the iliac crest including the anterior superior iliac spine (IL-I), the midpoint between the former and the apex of the iliac crest (IL-II), the apex of the iliac crest (IL-III), and the posterior superior iliac spine (IL-IV). The MAL was represented by the radius of the femoral head for IL-I in joint angles up to 10°, for IL-II up to 30°, for IL-III and IL-IV up to 60°, and by the length of the vertical line from the hip joint center to the vector from the origins to the insertion in larger joint angles.

torque produced by the PM, IL, and rectus femoris as the product of the PCSA and the MAL. The indicators were calculated for individual muscles, and the relative contribution was calculated as the ratios of indicators to the total hip flexion torque for the three muscles at various hip joint angles from extension to flexion (0°–90°).

Estimation of the relative rotation speed by contraction of the flexors

As an indicator of rotation speed by contraction of the PM, IL, and rectus femoris, the change of flexion angle ($\Delta\alpha$) due to the 1% shortening of the muscle fiber (ΔFL) was calculated from the muscle FL, pennation angle, and MAL (R) by the following equations under the specific condition that each muscle has the same muscle contraction speed:

$\Delta FL = FL \times 0.01$, and

$\Delta\alpha = \arctan(\Delta FL \times \cos\theta / R)$ (equation 1).

The equations were derived from the following geometrical reasoning. The shortening of the muscle length (ΔML) and the ΔFL were correlated with the following equation:

$$\Delta ML = \Delta FL \times \cos\theta$$

The ΔML was thought to flex the joint by the angle change $\Delta\alpha$ with the equation:

$$\Delta ML = R \times \tan\Delta\alpha$$

The two equations gave an equation from which equation (1) was derived:

$$\tan\Delta\alpha = \Delta FL \times \cos\theta / R$$

Data analyses

The morphometric and calculated values including PCSA were analyzed by one-way analysis of variance and multiple comparison tests (Tukey's honest significant difference test, Bonferroni). All values are presented as mean ± standard deviation. Statistical analyses were conducted using the IBM SPSS version 23 (IBM Corporation, Armonk, NY, USA), and the significance level for all tests was set at $P<0.05$.

Results

Anatomy of the iliopsoas and rectus femoris

The PM, with two heads arising from two different origins, originated as the superficial head from the intervertebral discs between T12 and L4 and the adjacent rims of the vertebral bodies, and as the underlying head from the costal process of L1–L5 and the twelfth rib, and converged to form the insertion tendon ending on the lesser trochanter of the femur. The IL, originating from the iliac fossa and the anterior inferior iliac spine, terminated mainly via the insertion tendon and partly directly without the tendon on the lesser trochanter. The psoas minor, originating from the vertebral body of T12 and L1 and inserting onto the iliac fascia at the iliopubic ramus, contributed little to the flexion of the hip joint. The rectus femoris originated at the anterior inferior iliac spine and the upper part of the acetabulum and inserted onto the tibial tuberosity through the patella.

The PCSA of the iliopsoas (PM, IL) and the rectus femoris was estimated from the morphometric data obtained from the isolated muscle specimens (Figure 2). The muscle FL of the iliopsoas (PM: 13.76 cm, IL: 11.10 cm) was longer than that of the rectus femoris (8.04 cm) ($P<0.01$). The PCSA of the rectus femoris (10.88 cm^2) was larger than that of the PM (5.45 cm^2) ($P<0.05$). The PCSA of the iliopsoas was comparable with that of the rectus femoris (Table 1).

MAL under different hip joint angles in the sagittal plane

In the hip extension position (0°), the MAL of the rectus femoris (35.0 ± 4.0 mm) was larger than that of PM and IL (22.9 ± 1.9 mm). When the hip joint was flexed, the MAL of the rectus femoris increased gradually in mild flexions to achieve a maximal value at 40° (50.4 mm) and decreased

Figure 2 Photographs of isolated muscle specimens of the iliopsoas and rectus femoris in the left-side and superficial views. PMi, psoas minor; PMa, psoas major; IL, iliacus; RF, rectus femoris

Table 1 Parameters of the mechanical architecture of the iliopsoas and rectus femoris

	Muscle mass(g)	θ(°)	FL(cm)	PCSA(cm^2)
Psoas major	81.4±47.4	6.3±2.1	13.76±1.19**	5.45±2.85*
Iliacus	84.1±47.1	7.9±2.6	11.10±1.74**	6.91±2.98
Rectus femoris	90.4±52.8	6.5±2.0	8.04±1.23**	10.88±6.00*

θ, pennation angle; FL, muscle fiber length; PCSA, physiological cross-sectional area
**P<0.01, *P<0.05

gradually in deep flexions up to 90°. The MAL of PM-I was slightly larger than that of PM-II between 60° and 90° and exhibited a similar pattern of change during hip flexions. The MAL of IL-I and IL-II remained mostly constant up to 10° and 30°, and thereafter in deep flexions, increased steeply up to 90°, exceeding that of the rectus femoris at the flexion between 40° and 60°. The MAL of IL-Ⅲ and IL-Ⅳ remained constant up to 50° and 60°, and increased steeply up to 90°, exceeding that of the rectus femoris at the flexion between 70° and 80° (Figure 3-A).

The MAL of the PM remained unchanged in moderate flexions up to 50° and thereafter increased steeply in deep flexions, exceeding that of the rectus femoris at the flexion between 60° and 70°. Conversely, the MAL of the IL increased gradually in deep flexions, exceeding that of the rectus femoris at the flexion between 60° and 70° (Figure 3-B).

Relative contribution of individual hip flexors to the maximal hip flexion torque

The relative contribution of the rectus femoris in the hip extension position at 0° (57.3%) exceeded that of the iliopsoas and increased gradually in flexions to an almost maximal value at 20°-40° (60.4%-62.3%); thereafter, it decreased drastically in deep flexions up to 90°, falling behind that of the iliopsoas at a flexion of approximately 60°-70°. The relative contribution of the IL in the hip extension position

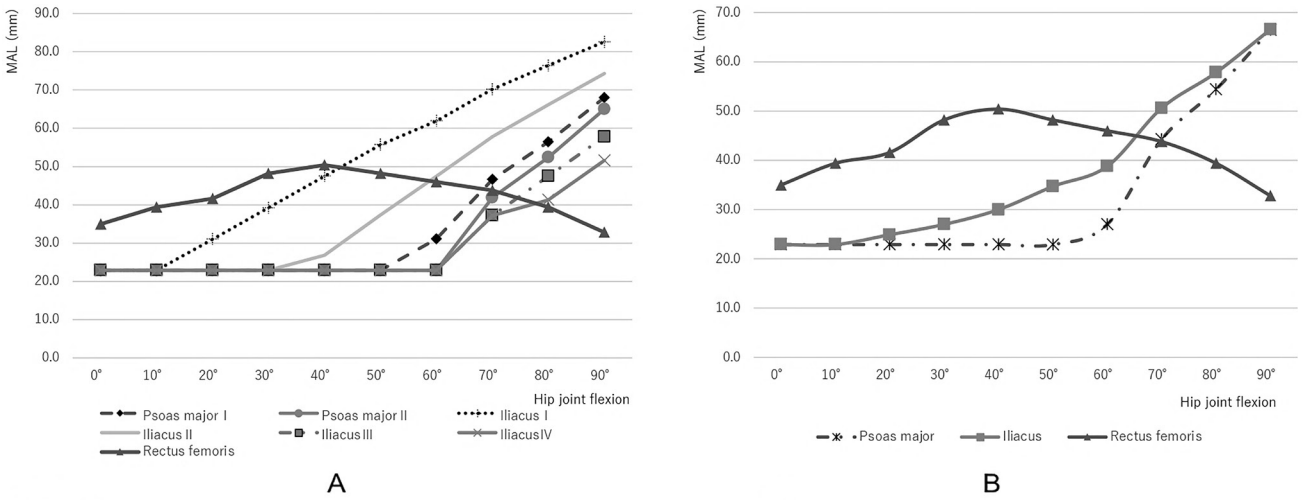

Figure 3 Moment arm length (MAL) under different hip joint angles on the sagittal plane.
A. MAL of the iliopsoas (PM-I·II, IL- Ⅰ-Ⅳ) and rectus femoris estimated in different hip joint angles.
The MAL of the rectus femoris increased gradually in mild flexions to reach a maximal value at 40° and decreased gradually in deep flexions up to 90°. The MAL of PM-I was slightly larger than that of PM-II between 60° and 90° and exhibited a similar pattern of change during hip flexions. The MAL of IL-I and IL-II almost remained constant up to 10° and 30°, and thereafter in deep flexions, increased steeply up to 90°, exceeding that of the rectus femoris at the flexion between 40° and 60°. The MAL of IL-Ⅲ and IL-Ⅳ remained constant up to 50° and 60°, increased steeply up to 90°, exceeding that of the rectus femoris at the flexion between 70° and 80°.
B. MAL of the iliopsoas and rectus femoris estimated in different hip joint angles.
The MAL of the rectus femoris was much larger than that of the iliopsoas in the hip extension position (0°). It increased gradually in mild flexion up to 40° and then decreased gradually. The MAL of the psoas major and iliacus remained unchanged in moderate flexions up to10° and 60°, and thereafter increased in deep flexions up to 90°, exceeding that of the rectus femoris at the flexion between 60° and 70°.

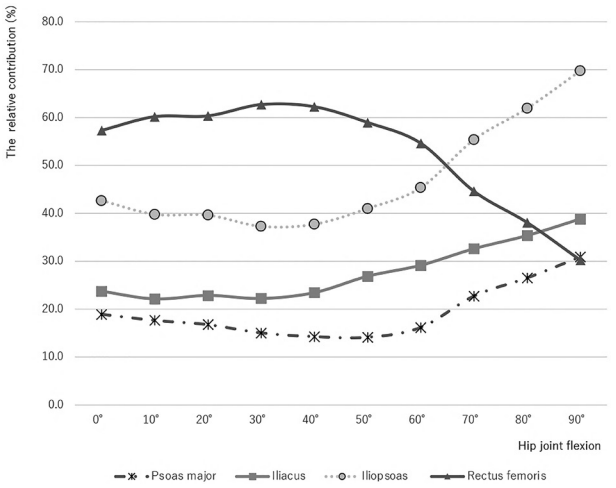

Figure 4 The relative contribution of the iliopsoas and rectus femoris to the maximal hip flexion torque.
The relative contribution of the rectus femoris to the maximal hip flexion torque was greater than that of the iliopsoas in mild flexion up to 60°, and its relative contribution decreased steeply in deep flexion. Moreover, the relative contribution of the iliopsoas to the torque increased sharply in the deepest flexions at 80° and 90°.

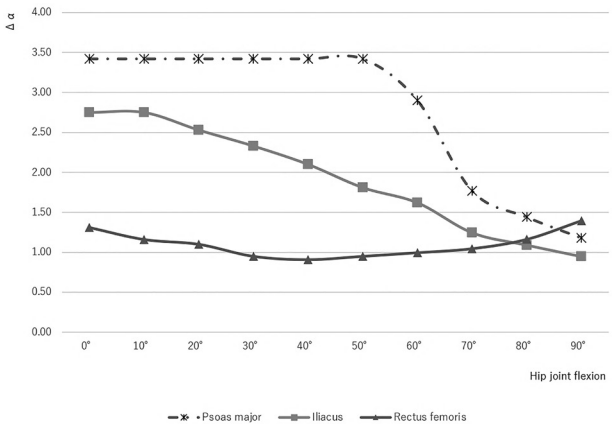

Figure 5 The relative rotation speed is represented by the change in flexion angle due to a 1% shortening of the muscle fiber.
The change of flexion angle ($\Delta\alpha$) of the psoas major remained constant in mild hip joint flexions up to 50° and decreased steeply up to 90°. $\Delta\alpha$ of the iliacus remained constant in hip joint flexions up to 10° and decreased gradually in flexions up to 90°. That of the rectus femoris decreased slightly in mild flexions up to 40° and thereafter increased slightly up to 90° to be at the same level as 0°.

at 0° remained unchanged in moderate flexions up to 40° and thereafter increased gradually in deep flexions up to 90°, exceeding that of the rectus femoris at the flexion between 80° and 90°. The relative contribution of the PM decreased gradually in flexions up to 50° and thereafter increased in deep flexions up to 90° to become the same level as the rectus femoris in the hip extension position at 90° (Figure 4).

Relative rotation speed by contraction of the flexors

In the hip extension position (0°), the relative rotation speeds of the PM and IL were 2.6– and 2.1–times swifter than that of the rectus femoris, respectively. The relative rotation speed of the PM remained constant in mild hip joint flexions up to 50°, and thereafter in deep flexions, decreased steeply up to 90°. The relative rotation speed of the IL remained constant in hip joint flexions up to 10° and decreased gradually in flexions up to 90°. Conversely, the relative rotation speed of the rectus femoris decreased slightly in mild flexions up to 40° and became approximately 90% compared with that at 0°; thereafter, it increased slightly up to 90° to become the same level as that at 0° (Figure 5).

Discussion

In this study, we evaluated the different action of the iliopsoas and the rectus femoris on the hip flexion by estimating their relative contribution to the maximal hip flexion torque and relative rotation speed, and found that the iliopsoas served as a rapid flexor, whereas the rectus femoris was a powerful flexor.

The joint torque or force is produced by several agonist muscles and determined by various factors including neural activities[34], muscle fiber types[35], and contraction speed[36]. For improving performance in athletic sports, it is important to understand the contribution of individual agonist muscles in producing torque in the hip joint. However, to the best of our knowledge, this has not been investigated properly to date. Hip joint flexion is exerted mainly by the iliopsoas and rectus femoris, and other accompanying muscles including the sartorius and adductor longus. The importance of the iliopsoas and rectus femoris as hip joint flexors is indicated by their larger PCSA (9.9 + 7.7 + 13.5 = 31.1 cm^2) than that of the sartorius and adductor longus (1.9 + 6.5 = 8.4 cm^2)[26]. The sartorius and adductor longus have much smaller PCSA values, and their force vectors deviate from the sagittal plane. Actually, Inai et al[37]. considered the iliopsoas

and rectus femoris as the hip flexors in a computer simulation study investigating the dynamics of hip flexors during sit-to-stand movements. A study on iliopsoas function reported that the iliopsoas was activated during increased hip flexion when the subjects moved from the standing position (0°; a fully extended position of the hip and knee joints) to one-leg flexion of the hip joint (90°)[18]. Furthermore, the contribution ratio of the four hip flexors (the iliopsoas, rectus femoris, tensor fasciae latae, and sartorius) to the flexion torque changes depending on the posture during the measurement of activities in these muscles[9]. However, the contribution ratios of the muscles to the flexion torque were not clarified in these studies. The relative contribution of the individual flexors to the joint torque could not be experimentally obtained either by measurement of joint torque or by EMG of the relevant muscles. The relative contribution of the individual flexors to the maximal joint torque could be estimated on a theoretical basis by calculating the indicator of maximal joint torque as the product of the PCSA and the MAL for the individual muscles. It is well known that the joint torque exerted by a muscle is the product of muscle tension and the MAL and that the PCSA is an index of the maximal exertion tension of the muscle[38]. To date, the PCSA and MAL have been measured separately in different studies. In this study, we measured both of them with cadaveric dissection for the first time, thus providing novel findings. The PCSA was calculated from morphometric parameters, such as the muscle volume, FL, and pennation angle, which could be accurately measured in the isolated muscle specimens[26,30-32].

The PCSA of a given muscle varied between the present and previous studies, especially because of the large individual variation in the muscle mass. In the present study, we calculated the ratio of the PCSA among the three muscles and its coefficient of variation (CV; standard deviation divided by the average) and found that the CV for the ratio of PCSA was quite small in contrast to the large CV for the PCSA, supporting the reliability of the estimation of the relative contribution of each muscle to the maximal flexion torque (Table 2).

The MAL of the iliopsoas and rectus femoris and its change under different hip joint angles have been calculated[39] using a method based on an interactive musculoskeletal modeling software and the lower limb model of Delp et al[40]. Their results based on model simulation were in good agreement with the results found for the skeletal specimens in the present study. The small and stable value for the iliopsoas in mild flexions up to 60° could be explained by the bend of the insertion tendon on the femoral head, and the steep increase in deep flexions could be the result of its distance from the head. The MAL of the rectus femoris was maximal at a hip flexion of 40°, where the two vectors from the origin (anterior inferior iliac spine) to the center of the hip joint and the insertion (upper edge of the patella) crossed vertically.

The present study revealed the main agonists of hip joint flexion as two functionally important parameters for the first time: the relative contribution to the maximal flexion torque and the relative rotation speed by muscle contraction. From these parameters, different functional properties of the iliopsoas and rectus femoris became apparent.

We also showed that the rectus femoris was a more powerful flexor than the iliopsoas in mild hip flexions up to 60° and a less powerful flexor in deep hip flexions, which is in agreement with the larger activities of the iliopsoas in deep hip flexions[18]. Regarding the relative contribution of the iliopsoas

Table 2 The PCSA of the psoas major, iliopsoas, and rectus femoris, and its ratio to the total amount

	PCSA		Ratio of PCSA	
	Average ± standard deviations (cm^2)	CV	Average ± standard deviations (%)	CV
Psoas major	5.45±2.85	0.52	23.58±4.45	0.19
Iliacus	6.91±2.98	0.43	30.74±5.88	0.19
Rectus femoris	10.88±6.00	0.55	45.68±8.10	0.18

PCSA, physiological cross-sectional area; CV, standard deviation divided by average.

The PCSA exhibited a significant individual variation, which is represented by the large value of the CV. However, the ratio of the PCSA was almost stable among individuals, which is indicated by the small value of the CV.

and rectus femoris to the maximal flexion torque, the rectus femoris plays a more important role in the shallow flexion of the hip joint during walking among the activities of daily living.

Conversely, it was also shown that the relative rotation speed by muscle contraction for the PM and IL was 3.6 and 1.9 times larger than that of the rectus femoris in mild hip flexions up to 50°, respectively. Regarding estimation of the rotation speed by contraction of the flexors, it was clarified that the relative rotation speed of flexion differs between the iliopsoas and rectus femoris muscles under the specific condition that each muscle has the same muscle contraction speed. It would be reasonable to argue that the iliopsoas is particularly important for sprinters, contributing significantly to leg swing speed. In addition, weak hip flection muscles pose a risk for falling, and the main cause of injury in people with weak hip flexion muscles is stumbling or falling while climbing stairs. Strengthening the iliopsoas makes it possible to lift the leg instantly, thereby preventing falls when climbing stairs or stepping over obstacles.

This study has some limitations. We did not measure the sarcomere length in each muscle; therefore, the FL for calculating the PCSA was not normalized by the ratio of the optimal and measured sarcomere length. Further refinements of the PCSA by normalizing the FL would be beneficial for achieving a more precise estimation of the relative contribution to the maximal torque and the relative rotation speed. In addition, muscle fibers include slow-twitch fibers (Type I), which have a slow contraction rate and are less likely to fatigue, and fast-twitch fibers (Type II), which have a high contraction rate and are prone to fatigue. In human muscle, slow- and fast-twitch fibers are present in different ratios. Lieber[24] indicated that the differences in muscle fiber types have little effect on exercise performance, although there are differences in the maximum contraction rates of Type I and Type II fibers. In the present study, the effect of different muscle fiber types on relative rotation speed was not considered. Further improvements that also consider the histological factors of the muscle fibers would be beneficial for achieving a more precise estimation of the relative contribution of each fiber type to the relative rotation speed.

In conclusion, the present study clarified the functional characteristics of the iliopsoas and rectus femoris based on the morphometric data from skeletal anatomical specimens and applied the results to the simple movement of hip flexion on a theoretical basis to provide a better understanding of the functional contribution of muscles experimentally to the actual movements of the hip joint. The results of the present study would provide useful information for the functional training of athletes and rehabilitation programs in the clinic. Future developments of this research technique would clarify the functional contribution of the individual muscles in complex joint movements.

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Author contributions

All authors have made substantial contributions to the manuscript. The details are as follows:

TK: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Project administration, Writing – original draft, Writing – review & editing

TT: Resources, Writing – review & editing, Supervision

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TS: Conceptualization, Methodology, Data curation, Visualization, Writing – review & editing, Supervision

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The authors declare that there are no conflicts of interest.

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Original Articles

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Degree of Differentiation of Esophageal Squamous Cell Carcinoma and Micrometastasis to Lymph Nodes

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Objectives: The goal of the study was to examine the relationships among micrometastasis, pathological degree of differentiation and survival in patients with esophageal squamous cell carcinoma (SCC).
Design: A single-center retrospective study of patients diagnosed with thoracic esophageal SCC.
Methods: Immunostaining using CK13 was carried out for all lymph nodes resected by radical esophagectomy with three-field lymphadenectomy. The relationships among micrometastasis to lymph nodes, degree of differentiation and survival were investigated.
Results: The 25 patients included 14 (56.0%) well-differentiated and 11 (44.0%) moderately differentiated cases. In multivariate analysis, well-differentiated cases were not related to micrometastasis (odds ratio (OR): 1.5, confidence interval (CI): 0.2–12, p=0.7). In multivariate analysis of survival, cases in pStage III or higher were likely to have shorter survival (hazard ratio (HR): 2.8, CI: 0.7–12, p=0.16), and those with micrometastasis also tended to have shorter survival (HR: 2.7, CI: 0.8–9, p=0.11)); however, well-differentiated cases were not significantly related to survival (HR: 1.5, CI: 0.4–5.5, p=0.5).
Conclusion: Micrometastasis to lymph nodes may be a prognostic factor even in advanced esophageal cancer. The degree of differentiation was not related to micrometastasis or survival.

Key words: outcomes, differentiation, esophageal squamous cell carcinoma, micrometastasis, lymph nodes

Introduction

Esophageal squamous cell carcinoma (SCC) has a poor prognosis, with recurrence in lymph nodes and distal metastasis found frequently within a few years after surgical resection[1)]. Some of these findings may be caused by micrometastases that cannot be detected by hematoxylin-eosin (HE) staining[2)]; that is, pathological, rather than clinical, metastasis. Many studies have examined micrometastases in esophageal cancer, particularly to lymph nodes, but these studies have mostly been limited to earlier stage cancer[3–6)]. Thus, micrometastasis to lymph nodes in advanced esophageal cancer is poorly understood.

The relationship between outcomes of esophageal cancer and pathological degree of differentiation has been widely investigated. In general, well-differentiated carcinoma has a better prognosis and poorly differentiated cases have a poor prognosis[7)]. However, the relationship between micrometastasis to lymph nodes and the histo-

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pathological degree of differentiation is unknown. One study showed more micrometastases in well differentiated esophageal carcinoma[5], but another found opposite results[8], and currently, no conclusion has been reached. Therefore, we examined this relationship and that of micrometastasis to lymph nodes with prognosis in patients with esophageal SCC, including cases of advanced cancer.

Methods

This study was approved by the clinical research committee of Juntendo University Hospital (E21-0056-H01). Informed consent was not required because of the retrospective study design. Twenty-five patients with esophageal cancer underwent esophagectomy in our department at Juntendo University Medical School from January to December 2000. The inclusion criteria were [1] SCC, [2] no preoperative chemotherapy, [3] three-field lymphadenectomy, [4] completion of follow-up, and [5] complete R0 resection. The exclusion criteria were >10 metastases to lymph nodes detected by conventional HE stain.

Surgery

All patients underwent esophagectomy with three-field lymphadenectomy by right thoracotomy and laparotomy, as reported previously9). All patients also underwent lymphadenectomy along the bilateral recurrent laryngeal nerves and around the supraclavicular area. The gastric tube was pulled up through the retrosternal route and a hand-sewn esophagogastrostomy was created in the neck.

Postoperative adjuvant therapy

Patients who were pathologically confirmed to have ≥3 lymph node metastases received two courses of postoperative adjuvant therapy with docetaxel, cisplatin and 5-fluorouracil[10].

Immunostaining

For histopathological examination of resected lymph nodes, samples were subjected to formalin fixation and paraffin embedding. Five neighboring tissue sections (3 μm) were prepared from each slice and 3 central sections were used for staining. After deparaffinizing each section, antigen retrieval was carried out by autoclave treatment (1.2 atm, 10 min). Immunostaining was performed using anti-cytokeratin13 Ks 13.1 mouse monoclonal antibody (CK13, American Research Products) diluted 20 times with antibody diluent (1% BSA/PBS) using automated staining equipment (Ventana NX System, Ventana). After immunostaining, background staining was performed with Mayer's hematoxylin for microscopic examination. Of the cells within the lymph node capsules, nucleated cells with evenly cytokeratin-stained cytoplasm were defined as metastasis-positive.

Clinicopathologic parameters

Data for clinicopathologic parameters, including tumor stage by TNM staging (ver. 8)[11], were obtained retrospectively from a hospital database. TNM staging reflects the result of HE stain. The degree of differentiation was evaluated using most parts of the tumor.

Statistical analysis

Comparisons of two groups were performed by chi-square test. Survival curves were estimated with the Kaplan-Meier method and significant differences in survival rate were analyzed by log-rank test. Multivariate analysis was performed using logistic regression analysis for categorical data and Cox regression analysis for survival. Significance was defined as $P<0.05$ in all analyses. Due to the small number of cases, a result with $P<0.2$ was considered not to be significant, but to show a tendency. All calculations were performed using IBM SPSS Statistics ver. 23.0.

Results

The characteristics of the 25 patients in the study are shown in Table 1. The most common disease was middle thoracic esophageal cancer, pT3, pN1, pStage III. There were 14 (56%) well-differentiated, 11 (44%) moderately differentiated, and no poorly differentiated cases. Intramural metastasis was found in resected specimens in 4 patients.

Conventional lymph node metastases

A total of 2,915 lymph nodes were collected from the 25 patients. The median number of lymph nodes per patient was 116. In histopathologic examination using HE stain, metastases were detected

Table 1 Patient background

Variable		Number of patients	Micrometastasis-positive	Micrometastasis-negative	P
Sex	Male/Female	23/2	8/2	15/0	0.15
Site	Ut/Mt, Lt	3/22	1/9	2/13	0.654
Age	<60 / ≥60	12/13	6/4	6/9	0.284
Depth	pT1/pT2-pT3	11/14	2/8	9/6	0.048
Lymph node metastasis	pN0-N1/pN2-N3	14/11	2/8	12/3	0.003
Advanced stage	pStage0-Ⅱ/pStageⅢ-Ⅳ	11/14	2/8	9/6	0.048
Differentiation	well/mod	14/11	8/2	6/9	0.048
Lymphovascular invasion	ly0-2/ly3	20/5	5/5	15/0	0.002
Venous invasion	v0-1/v2	15/10	3/7	12/3	0.012
Intramural metastasis	IM0/IM1	21/4	6/4	15/0	0.008

Ut: upper thoracic esophagus, Mt: middle thoracic esophagus, Lt: lower thoracic esophagus, T: tumor, N: lymph node, well: well-differentiated squamous cell carcinoma, mod: moderately differentiated squamous cell carcinoma, ly: lymphovascular invasion, v: venous invasion, IM: intramural metastasis

Table 2 Histopathologic factors and degree of differentiation

	Well differentiated	Moderately differentiated	p
T1/T2-3	2/12	9/2	0.001
N1/N2-3	3/11	7/4	0.032
Micrometastasis(+)/micrometastasis(-)	8/6	2/9	0.048
pIM0/pIM1	10/4	11/0	0.053
ly0-2/ly3	10/4	10/1	0.085
v0-1/v2	5/9	10/1	0.005

T: tumor, N: lymph node, IM: intramural metastasis, ly: lymphovascular invasion, v: venous invasion

in 70 lymph nodes in 17 patients.

Micrometastasis to lymph nodes

In immunostaining with CK13, one micrometastasis was detected in one patient without evidence of lymph node metastasis on HE stain, and 16 micrometastases were detected in 9 patients who were determined to be metastasis-positive by HE stain. Therefore, 10 patients were evaluated as lymph node micrometastasis-positive. The main tumors stained positive for CK13 in all 25 cases. All lymph nodes that were found to be metastasized by HE stain also stained positive with CK13.

Relationships between micrometastasis and pathological factors

The micrometastasis-positive group included many cases with high pT and pN, advanced pStage, lymphatic and vascular invasion, and well-differentiated carcinoma (Table 1). A comparison based on differentiated types showed that well-differentiated cases were likely to have more advanced tumors (Table 2). To investigate the factors related to micrometastasis, logistic regression analysis was performed with pStage and degree of differentiation as independent variables and micrometastasis as the dependent variable. This analysis showed a tendency for cases in pStage III or higher to be more likely to be micrometastasis-positive (odds ratio (OR): 6.6, confidence interval (CI): 0.8-54, p=0.08). The OR for well-differentiated tumors was 1.5 (CI: 0.2-12), but the p value of 0.71 indicated that the relationship between degree of differentiation and micrometastasis was not significant (Table 3).

Long-term survival

Patients with micrometastasis to lymph nodes had shorter survival compared to those without micrometastases (p=0.002; Figure 1). There was

Table 3 Logistic regression analysis of relationships between micrometastasis and pathological factors (degree of differentiation and Stage)

		Odds ratio	Confident intervals	p
Degree of differentiation	Well vs. moderate	1.5	0.2 – 11.5	0.71
Stage	III, IV vs. I, II	6.6	0.8 – 55	0.079

also a significant difference in survival based on the degree of differentiation (p=0.031; Figure 2). However, when pStage, micrometastasis, and degree of differentiation were input as independent variables in a Cox proportional hazard model, all three factors were not significant (pStage III or higher, hazard ratio (HR): 2.8, CI: 0.7–12, p=0.16; micrometastasis, HR: 2.7, CI: 0.8–9, p=0.11; well-differentiated, HR: 1.5, CI: 0.4–5.5, p=0.5). Based on the p-values, pStage III or higher and micrometastasis showed a tendency to be related to survival, but degree of differentiation did not do so (Table 4).

Discussion

The first finding in this study is that micrometastasis to lymph nodes is not related to the degree of differentiation in esophageal SCC. There are contradictory findings for the relationship of micrometastasis with the degree of differentiation in esophageal cancer. In our study, micrometastasis was associated with the degree of differentiation in univariate analysis and well-differentiated cancers were likely to have micrometastasis. However, this was not shown in multivariate analysis. We speculate that the results in univariate analysis may be due to bias since patients with well-differentiated tumors tended to have higher stage disease in this cohort.

Secondly, micrometastasis to lymph nodes was found to be a likely prognostic factor, even for locally advanced esophageal cancer. This might be a new finding because most previous studies have examined patients with early disease[3–6], but this finding is also consistent with previous results for various carcinomas, including esophageal carcinoma. On the other hand, the degree of differentiation was not a prognostic factor in multivariate

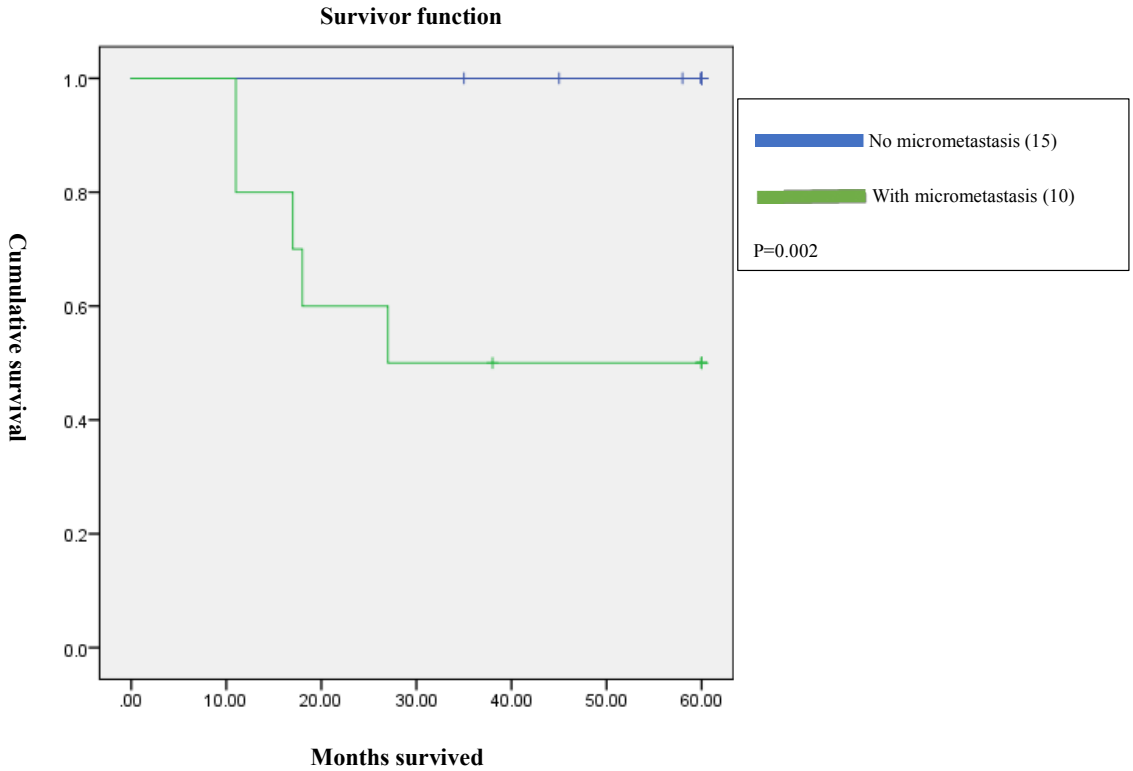

Figure 1 Survival curves for patients with and without micrometastasis. There was a significant difference in survival rate between those with and without micrometastasis (p=0.002).

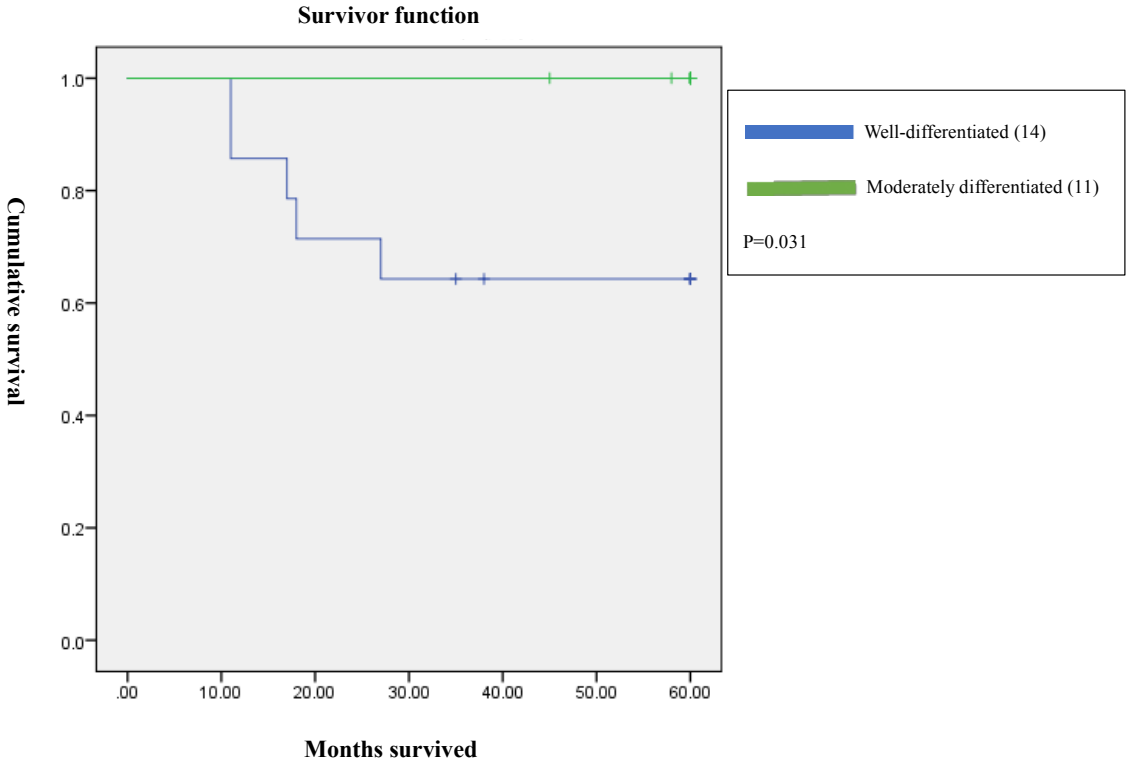

Figure 2 Survival curves based on extent of differentiation. There was a significant difference in survival rate between differentiation grades (p=0.031).

Table 4 Multivariate Cox regression analysis for degree of differentiation, Stage and micrometastasis

		Hazard ratio	Confidence intervals	p
Degree of differentiation	Well vs. Moderate	1.5	0.4 – 5.5	0.51
Stage	III, IV vs. I, II	2.8	0.7 – 12	0.16
Micrometastasis	Positive vs. Negative	2.7	0.8 – 9.1	0.11

analysis, which is inconsistent with previous studies showing that well-differentiated esophageal cancer is associated with good survival[7]. This may be attributable to a difference of definitions between Japan and Western countries, since the degree of differentiation is based on the dominant type in Japan, but on the poorest type in Western countries. For example, when the well-differentiated region is dominant and the moderately differentiated part is small in esophageal cancer, the Japanese pathological diagnosis is well-differentiated, but the Western diagnosis would be moderately differentiated. Furthermore, our small cohort did not include any poorly differentiated cases, and this may also have affected the results.

There are several methods for diagnosis of micrometastases: immunostaining of lymph nodes, as performed in this study; lymph node detection by reverse transcription-polymerase chain reaction (RT-PCR); and immunostaining or RT-PCR using peripheral blood. One problem with use of resected lymph nodes is that the results are available only after surgery. Adjuvant therapy after esophageal cancer resection is not the standard in Japan, and there is no effective evidence-based adjuvant treatment, even if a poor prognosis is predicted. Recently, however, it has been shown that administration of nivolumab after esophagectomy following preoperative chemoradiotherapy leads to an improved prognosis in patients without pathological complete response (pCR)[12], and this treatment has been covered by health insurance in Japan from 2021. Micrometastasis-positive patients might be eligible for adjuvant therapy even if their carcinoma is pN0 or pN1.

This study has some limitations. First, it was not

prospective and the number of patients was small. Second, in our small cohort, there were no cases of poorly differentiated esophageal cancer, and the analysis might have been affected by this biased population.

In conclusion, the results of this study suggest that micrometastasis to lymph nodes may be a prognostic factor, even in advanced esophageal cancer. The degree of differentiation was not related to micrometastasis or survival. Further studies are needed with more patients and addition of patients with poorly differentiated SCC to examine the underlying mechanisms.

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Author contributions

TAsakura performed data analysis and wrote the first draft of the manuscript.

HT corrected and approved the manuscript.

TM, AA contributed to material on pathology.

TAndo provided advice on micrometastasis and contributed to writing the manuscript.

NT, MT, YK made significant changes in revision of the manuscript.

All authors read and approved the final manuscript.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Original Articles

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Clinical Significance of Circulating Tumor Cells in Patients with Esophageal Cancer

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Objective: In recent years, circulating tumor cells (CTCs) have attracted attention for prediction of metastasis in breast, prostate, and colon cancers. This study aimed to investigate whether detection of CTCs could be prognostic factor in esophageal cancer.
Methods: This study involved 38 patients treated at Juntendo University from May 2010 to April 2013 who provided consent. CTCs were measured using CellSearch® system in preoperative peripheral blood. Clinicopathological parameters and prognostic factors were retrieved from our medical records.
Results: CTCs were detected in 6 of 38 patients (15.8%). Among patients' characteristics and clinicopathological features, CTC-positive group had higher serum SCC levels and tended to have more advanced cStages than the CTC-negative group. The CTC-negative group showed better survival curves than CTCs positive-group in both overall survival (OS) and disease-free survival (DFS) although the differences were not statistically significant. CTCs positivity has a possibility to be prognostic marker according to multivariable analysis of OS and DFS.
Conclusion: Although this study has some limitations, our results suggest that CTCs in preoperative peripheral blood has potential to be a prognostic marker for esophageal cancer.

Key words: esophageal cancer, hematogenous metastasis, circulating tumor cells, cellsearch®

Introduction

Among various cancers, approximately 25,920 new esophageal cancer cases are reported in Japanese national surveillance of 2018[1], and this number is steadily increasing[2]. Lymph node metastasis is the most common metastasis in resectable esophageal cancer; however, recurrence occurs in various sites. Of these, distant recurrences occur frequently and define prognosis after radical surgery with extensive lymph node dissection.

We sometimes encounter patients whose tumor marker levels in peripheral blood had increased before recurrence, or metastasis was detected by diagnostic imaging such as computed tomography, ultrasonography, magnetic resonance imaging, and positron emission tomography. Thus, more sensitive modalities are expected in clinical practice to detect the so-called "micrometastasis." One of these micrometastases are circulating tumor cells (CTCs). In this decade, immunohistochemical methods and polymerase chain reaction methods have been used to verify the presence of CTCs in peripheral blood in advanced cancers; however, there is no established detection method[3].

Recent studies have shown CTCs in peripheral blood of metastatic patients might be suggest poor prognosis[4-6], and if CTC detection becomes possible in clinical practice, it will be useful for assessing a prognosticator and predicting early therapeutic effects. Although the clinical significance of CTC detection technology had not been established,

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CellSearch® system (MENARINI, Itary) has good reproducibility and can detect even a single CTC in a 7.5-ml peripheral blood sample[7]. In breast, colorectal, and prostate cancers, CTC counts before treatment and after the initial therapy correlate strongly with progression-free survival and overall survival (OS), moreover, therapeutic effects and prognosis can be predicted by measuring the CTCs count[4–9]. As a result, the CellSearch® system has been approved by the United States Food and Drug Administration (FDA) to test for CTCs in breast, prostate, and colorectal cancers. This study aimed to investigate whether detection of CTCs with the CellSearch® system could similarly be useful to predict prognosis in esophageal cancer.

Methods

Patients

This study involved 38 esophageal cancer patients treated at the Juntendo University Hospital Department of Esophageal and Gastroenterological Surgery from May 2010 to April 2013. All patients had been pathologically diagnosed with esophageal cancer before treatment. The exclusion criteria are as follows: (1) multiple primaries (multicentric esophageal cancer are included); (2) non primary cases; (3) history of any cancer within 5years.

Written informed consents were obtained from all enrolled patients before this study. This study was approved by the Ethical Committee of Juntendo University Hospital (No.12-80).

Clinicopathological data were retrospectively retrieved from our database and electronic medical records. Tumor stage was assessed according to the International Union Against Cancer (UICC) TNM classification 7th edition for esophageal cancer[10] from findings of gastrointestinal endoscopy, upper gastrointestinal series, computed tomography, and endoscopic ultrasound.

Measurement of Circulating Tumor Cells

A CellSearch® system epithelial cell kit was used to detect rare CTCs in whole blood by immunomagnetic separation. The kit contains a magnetic bead-based capture reagent and an immunofluorescent staining reagent, and the magnetic beads contain nanoparticles with magnetic cores surrounded by a polymer layer coated with an epithelial cell adhesion molecule (EPCAM) antibody to enrich CTCs. After enrichment and concentration using immunomagnetic separation, staining reagents are added to detect CTCs. Anti-CK-PE is specific for the intracellular protein cytokeratin (specific to epithelial cells), a nucleic acid stain (4′,6-diamidino-2-phenylindole [DAPI]) stains the cell nuclei, and anti-CD45 and APC react specifically with leukocytes.

The reagent and sample mixture are placed in a cartridge that sits in a MagNest® holder that generates a magnetic field using the CellTracks AutoPrep system (MENARINI, Itary). Magnetically labeled epithelial cells move to the surface of the cartridge due to the strong magnetic field generated by the MagNest®, the fluorescent images are captured by the Cell Tracks® Analyzer II, and candidates stained with both CK-PE and DAPI inside the cartridge are displayed. Images are presented in a gallery format for the final cell classification. The images are classified as tumor cells based on morphology and phenotype (EPCAM+, CK+, DAPI+, and CD45−).

A 20-ml blood sample was collected before all treatment, and 10 ml was distributed among two Cell Save storage tubes. Subsequently, 7.5 ml of the 10 ml blood sample was transferred to a conical test tube, 6.5 ml of diluent was added, and the conical test tube was capped and mixed by inverting five times. The sample was centrifuged at 800 rpm for 10 min with the centrifuge brake released. The sample was placed in the CellTracks AutoPrep device of the CellSearch® system for processing within 1 hour.

The CellTracks® AutoPrep system specifically isolates and extracts epithelial cells from the many cells in the blood using magnetic particles that consist of antibodies for EPCAM bound to iron nanoparticles. The isolated epithelial cells are bound by a fluorescently labeled cytokeratin monoclonal antibody, and the nuclei are stained with DAPI fluorescent DNA stain. Similarly, leukocytes are bound by fluorescently labeled CD45 antibodies to distinguish them from CTCs. The CTC reaction solution is transferred into a cartridge that sits in a device with a fixed magnet called the MagNest and then placed in the CellTracks Analyzer II® of the CSS to analyze the test results. The magnetic force generated by the magnet in the MagNest moves the CTCs captured by the ferrofluid to the top of

the cartridge. The data of the fluorescent colors that appear on the upper surface of the cartridge are processed into fluorescence images for analysis and assessment. We defined CTC positive when CTC count is at least one in 7.5 ml of blood sample.

We measured serum tumor makers (cytokeratin fragment [CYFRA], squamous cell carcinoma antigen [SCC], carbohydrate antigen 19-9 [CA19-9], and carcinoembryonic antigen [CEA]) of same samples. Blood samples for CTCs and tumor makers were collected from first visit of our department to beginning of any treatment.

Statistical Analyses

All statistical analyses were performed using IBM SPSS advanced statistics ver.25. The chi-square test was applied for the differences of patients' characteristics and CTCs status between the CTCs-positive and the CTCs-negative groups. Serum CYFRA, CA19-9, SCC and CEA levels are divided into two groups of over and within normal range. Differences were considered significant at p-value <0.05. Values are expressed as median (range).

OS and disease-free survival (DFS) rates were calculated using the Kaplan–Meier method, and univariate analyses were performed by the Log-rank test. The DFS was defined as duration (days) from the date of surgery (or the first treatment day of chemotherapy or chemoradiotherapy patients) to the first relapse of cancer or death from any cause. Cox hazards regression analysis

Table 1 Patients' characteristics

Number of cases	38 cases	
Age	66 (47-84) years old	
Sex	Male	33 (86.8%)
	Female	5 (13.2%)
Treatment	Surgery	35
	Esophagectomy with three(two) -fields lymph node dissection	28 (73.7%)
	Laryngectomy with cervical esophagecto-my	1 (2.6%)
	Endoscopic treatment	6 (15.8%)
	Chemotherapy alone/ Chemoradiotherapy	3 (7.8%)
Primary tumor location	Ce	4 (10.5%)
	Ut	7 (18.4%)
	Mt	14 (36.8%)
	Lt	13 (34.2%)
Predominant Histological type	Well-differentiated SqCC	10 (26.3%)
	Moderately differentiated SqCC	21 (55.3%)
	Poorly differentiated SqCC	2 (5.3%)
	Others (adenocarcinoma, Basaloid carcinoma)	5 (13.2%)
cT classification*	cT1	15 (39.5%)
	cT2	4 (0.5%)
	cT3	15 (39.5%)
	cT4	4 (10.5%)
Clinical stage *	I	15 (39.5%)
	II	5 (13.2%)
	III	14 (36.8%)
	IV	4 (10.5%)

* UICC TNM Classification of Malignant Tumors, 7th ed

Ce: Cervical esophagus, Ut: Upper thoracic esophagus, Mt: Middle thoracic esophagus, Lt: Lower thoracic esophagus
SqCC: Squamous cell carcinoma

was performed to evaluate the effect of CTCs positivity and other parameters to OS and DFS. In Cox hazards regression analysis, cTMN stages are divided into the cStageI +II and III+IV groups.

Results

The demographics of the patients in the entire cohort are shown in Table 1. Surgical treatments were performed in 29 patients (76.3%), but cohort also included patients undergoing endoscopic treatment, chemotherapy and chemoradiotherapy. Eight of 29 patients who underwent esophagectomy received neoadjuvant therapy.

Among these 38 patients, CTCs were detected in only 6 (15.7%) patients, the CTC counts were 1/ 7.5ml of blood sample in 4 patients, 2 in one patient, and 190 in one patient. Therefore, we divided the patients into the CTC-positive and CTC-negative groups. (Table 2). CTC-positive group had higher serum SCC level than the CTC-negative group (p= 0.014), and also CTCs-positive group tended to have more advanced cStage than CTCs-negative group (p=0.055). There were no significant differences between these two groups in other clinicopathological factors.

Regarding survivals, the CTCs-negative group showed better survival curves than CTCs positive-group in both OS and DFS, however the differences were not statistically significant (Figure 1 and 2). In multivariate analysis, we chosen CTCs status, cStage, serum SCC level and CEA level as independent variables based on p-value of less than 0.2. Cox hazards regression model showed that CTCs status was likely to be a prognostic factor, but not statistically significant (OS: Hazard Ratio (HR) =0.358, 95% confidence interval (CI) 0.122-1.502, p =0.062, DFS: HR=0.358, 95% (CI) 0.152-2.323, p =0.455), as shown in Table 3A and 3B .

Discussion

In this study, we investigated the relationships between CTCs and survivals. We could show that patients without CTCs had better survival than those of CTCs positive, however the difference was not statistically significant. In multivariate analysis regarding OS and DFS, we were able to demonstrate that CTCs positivity has a possibility to be prognostic marker. We speculated that these discrepancies might be from small sample size and the heterogeneity of patients' background. Actually, our enrolled patients included those with early disease that can be treated by endoscopy and those with distant metastasis. We assume that the differences in survivals between CTCs positive and

Table 2 Comparison of CTC-positive and CTC-negative cases

Clinicopathological factors	Variables	CTC-negative	CTC-positive	p-Value
Sex	Male / Female	28/ 4	5/ 1	0.788
Age		66.0	67.0	0.110
Main treatment	Operation/ ESD/ CRT/ other	26/ 4/ 2/ 0	2/ 2/ 1/ 1	0.630
Primary tumor location	Ce/ Ut/ Mt/ Lt	3 /6/ 11/ 12	1/ 1/ 3/ 1	0.727
Predominant Histological type	Well/ Mod/ Poor/ other	9/ 18/ 2/ 3	1/ 4 /0/ 1	0.732
cT classification*	T1/ T2/ T3/ T4	13/ 4/ 13/ 2	2/ 0/ 2/ 2	0.257
cN classification*	N0/ N1/ N2/ N3	13/ 7/ 10/ 2	2/ 0/ 2/ 2	0.171
cStage*	I/ II/ III/ IV	12/ 4/ 14/ 2	2/ 0/ 1/ 3	0.055
CYFRA	high/ normal range	3/ 28**	2/ 4	0.325
CA19-9	high/ normal range	2/ 30	1/ 5	0.385
SCC	high/ normal range	3/ 28**	3/ 3	0.014
CEA	high/ normal range	10/ 20**	0/ 6	0.096

ESD: Endoscopic submucosal resection, CRT: Chemoradiotherapy

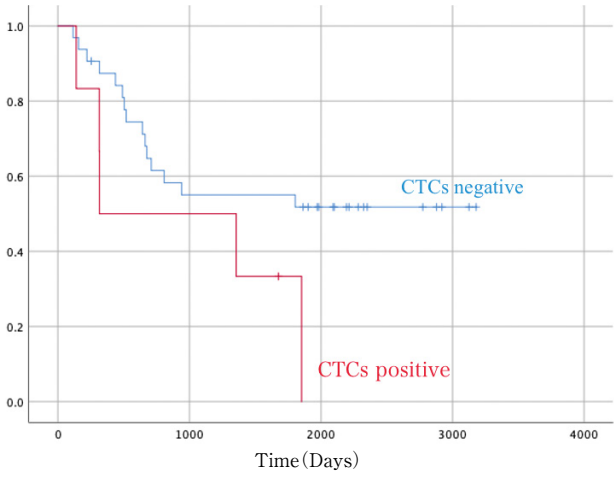
Ce: Cervical esophagus, Ut: Upper thoracic esophagus, Mt: Middle thoracic esophagus, Lt: Lower thoracic esophagus

Well: Well differentiated Squamous cell carcinoma, Mod: Moderately differentiated Squamous cell carcinoma, poor: poorly differentiated Squamous cell carcinoma

CYFRA: cytokeratin fragment, CA19-9: carbohydrate antigen 19-9, SCC: squamous cell carcinoma antigen, CEA: and carcinoembryonic antigen

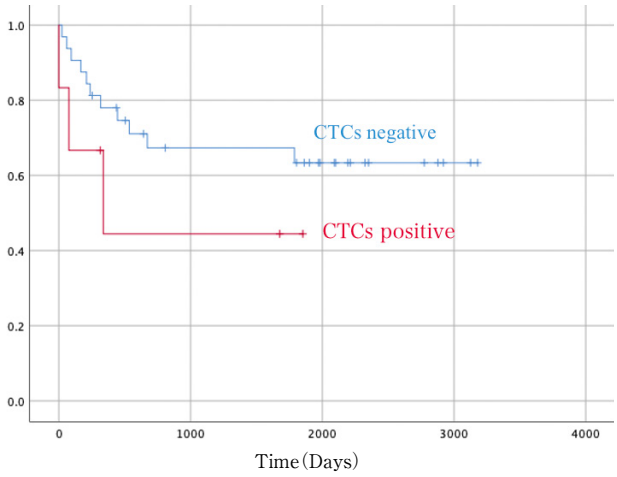
* UICC TNM Classification of Malignant Tumors, 7th ed

**not measured in some cases



Log-rank test p= 0.082

Figure 1 Overall survival (OS) in the CTC-negative and -positive groups



Log-rank test p= 0.240

Figure 2 Disease-free survival (DFS) in the CTC-negative and -positive groups

Table 3 Long-term outcome and CTCs

A) Overall survival

Clinicopathological factors	Variables	p-Value	Exp(b)	95% CI Lower	95% CI Upper
CTCs	Positive/ negative	0.062	0.358	0.122	1.502
cStage*	I+II/ III+IV	0.006	2.237	1.259	3.973
CEA	(continuous)	0.519	1.016	0.968	1.067
SCC	(continuous)	0.647	1.006	1.259	3.973

B) Disease-free survival

Clinicopathological factors	Variables	p-Value	Exp(b)	95% CI Lower	95% CI Upper
CTCs	Positive/ negative	0.455	0.594	0.152	2.323
cStage*	I+II/ III+IV	0.021	11.725	1.458	94.266
CEA	(continuous)	0.325	1.026	0.975	1.079
SCC	(continuous)	0.802	1.003	0.978	1.029

SCC: squamous cell carcinoma antigen, CEA: carcinoembryonic antigen
* UICC TNM Classification of Malignant Tumors, 7th ed

CTCs negative groups would be clear if patients' backgrounds were limited to some extent.

In addition, we investigated the relationships between the detection of CTCs using the CellSearch® system and clinicopathological factor for patients with esophageal cancer. Although the CellSearch® system is approved by the FDA for some adenocarcinomas (breast, colorectal, and prostate cancers)[4–9], little has been reported on SqCC. The CTCs detection rate in esophageal squamous cell carcinoma is so low that few studies have been conducted in this area[11–13]. Actually, the CTCs positivity rate in the present study of esophageal cancer was low, 15.7%. This could be due to the distribution of the patients' characteristics with relatively early-stage cancers. Among this cohort, 39.5% had a depth of T1, 39.4% had no lymph node metastasis, and 31.5% had cStage I cancer.

According to our results, CTCs status was significantly related to the serum SCC level. SCC is widely found in epithelial cells and known as tumor maker of squamous cell carcinoma, not only esophagus but also lung, head and neck and others, known to associated with metastasis and poor prognosis[14–16]. In addition, in some previous studies, SCC mRNA in lymph nodes or peripheral blood

were used as a marker of micrometastasis of squamous cell carcinoma. Thus, CTCs counts, as micrometastasis, might be related with SCC level.

This study has some limitations. First, only a small number of patients were enrolled this study. Second, this study utilized a retrospective design. Recently, the sensitivity of CTCs detection reported to improve in other cancers[17, 18], therefore, we would consider large sample sizes to the analysis in future.

In conclusion, our results suggest that CTCs in preoperative peripheral blood has possibility to prognostic marker of esophageal cancer. Further studies are needed to confirm this finding.

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Author contributions

HK corrected blood samples, interpreted the patient data, and was a major contributor in writing the manuscript. MT and YK recruited patients. The article was revised by MN and the final manuscript has been approved by all authors.

Conflicts of interest statement

The authors declare that they are no conflicts of interest.

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Original Articles

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Semiautomatic Treatment Planning for the Field-in-field Technique in Whole Brain Irradiation

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Objectives: In radiation therapy, the field-in-field (FIF) technique is used to prevent the administration of unnecessarily high doses to reduce toxicity. Recently, the FIF technique has been used for whole brain irradiation (WBI). Using the FIF technique, the volume that receives a higher than prescribed dose (hotspot) can be largely reduced; however, the treatment planning requires time. Therefore, to reduce the burden on the treatment planners, we propose a semiautomatic treatment planning method for the FIF technique.

Methods: In the semiautomatic FIF technique, hotspot regions in a treatment plan without the FIF technique are identified three-dimensionally, and beams with blocks that cover the hotspot regions using a multileaf collimator (sub-beams) are automatically created. The sub-beams are added to the original plan, and weights are assigned based on the maximum dose of the original plan to decrease the doses in the hotspot regions. This method was applied to 22 patients previously treated with WBI, wherein treatment plans were originally created without the FIF technique.

Results: In the semiautomatic FIF plans, the hotspots almost disappeared. The dose to 95% of the volume and the volume receiving at least 95% of the prescribed dose in the planning target volume decreased by only 0.3% ± 0.2% and 0.0% ± 0.1%, respectively, on average compared with those in the original plan. The average semiautomatic FIF processing time was 28 ± 4 s.

Conclusions: The proposed method reduced the hotspot regions with a slight change in the target coverage.

Key words: automation, radiotherapy treatment planning, whole brain irradiation

Introduction

Whole brain irradiation (WBI) is one of the main treatments for patients with brain metastases and is also performed for prophylactic cranial irradiation as an adjuvant therapy for patients with small cell lung cancers[1–3]. WBI is usually performed using opposed lateral fields. Treatment with opposed lateral fields generates a hotspot in the frontal lobe, where the dose is considerably higher than the prescribed dose[2, 4]. Recently, the uniformity of the target dose has been improved by introducing advanced techniques, such as intensity-modulated radiation therapy. The International Commission on Radiation Units and Measurements (ICRU) recommends that the dose in the planning target

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volume (PTV) should be 95%–107% of the prescribed dose[5]. In this situation, a method to reduce hotspots should be used, even in WBI, to reduce the risk of cognitive impairment as much as possible.

The field-in-field (FIF) technique has been used to reduce unnecessarily high doses[6,7]. To apply the FIF technique to WBI, several beams are added to the opposed lateral fields as sub-beams. In the sub-beams, the hotspot regions are blocked to reduce the dose in these regions[7–9]. In this technique, additional time is required to create the sub-beams manually. If treatment planning using the FIF technique is automated, the burden of treatment planners is greatly reduced without compromising the quality of the treatment plans[10,11].

Several studies have been conducted on automatic planning for conventional techniques, including the FIF technique[11–14]. Kim et al. retrieved Digital Imaging and Communications in Medicine (DICOM) data from a treatment planning system (TPS) and created FIF plans for breast-conserving therapy[12]. Yu et al. developed an automatic multileaf collimator shaping technique for WBI using deep learning[14]. They adopted a simple two-opposing-lateral-field technique, and therefore, obtained the relatively high average max dose of approximately 110%.

In this study, we propose a semiautomatic treatment planning method for the FIF technique for WBI using an application programming interface provided by a TPS manufacturer without outputting DICOM data outside the TPS.

Materials and Methods

Overview of the proposed method

In the FIF technique for WBI, we developed an automatic technique for creating sub-beams, and adjusting beam weights to make hotspot regions disappear. Because we only automated the sub-beams creation and weight adjusting for the FIF technique, we describe our method as semiautomatic. In an automatic planning, whole planning process from the creation of main beams will be automated. The hotspot regions were defined as regions receiving dose above a predefined threshold D_{th}. Figure 1 shows an outline of the proposed method. The input of the method (an original treat-

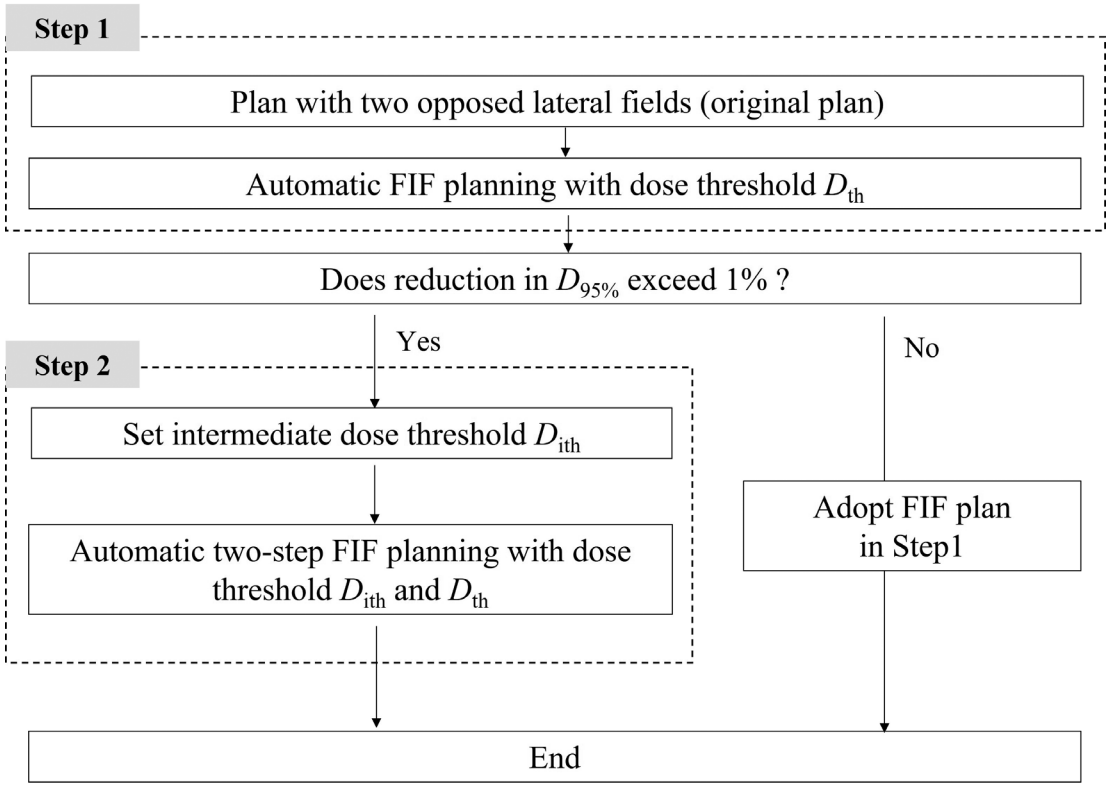

Figure 1 Flowchart of the semiautomatic field-in-field (FIF) technique
D_{th} is the dose threshold for the hotspot regions and fixed at 105% of the prescribed dose at the isocenter in this study. $D_{95\%}$ is the dose covering 95% of the PTV. D_{max} is the maximum dose in the original plan. D_{ith} is the intermediate threshold defined as $D_{ith}=(D_{max}+D_{th})/2$.

ment plan) was a conventional treatment plan using two-opposing-lateral fields for WBI, which was created in a TPS (Eclipse version 11.0; Varian Medical Systems, USA). The method consists of two steps: in Step 1, an algorithm for creating sub-beams and adjusting beam weights is applied to the original treatment plan and an FIF plan with two sub-beams is created with a predefined threshold D_{th} for hotspot regions; and in Step 2, the dose index of the PTV in the FIF plan is evaluated to determine whether further adjustment is required. When the reduction in the $D_{95\%}$ which represents the dose covering 95% of the PTV in Step 1 exceeds 1%., FIF treatment planning with four sub-beams is performed to alleviate the reduction of the $D_{95\%}$.

Steps 1 and 2 were implemented in a research version of the TPS (Eclipse, version 13.7; Varian Medical Systems, USA) using the Eclipse Scripting Application Programming Interface (ESAPI) and automatically performed.

The programming language used in the ESAPI was C# (Microsoft Corporation, USA). The research version of Eclipse (13.7) was used because Eclipse version 11.0 did not allow a change of a treatment plan using ESAPI. We retrospectively applied the automatic FIF script to the treatment plans of patients treated using the two-opposing-lateral-field technique.

Automatic FIF technique

FIF plan in Step 1

In the original treatment plan, hotspot regions that received a dose above D_{th} were identified in the three-dimensional (3D) dose distribution calculated in the TPS. The two beams in the original plan (main beams) were duplicated as sub-beams, which initially had the same multileaf collimator (MLC) positions as the main beams and no beam weights. In the FIF technique, the fields of sub-beams are shaped to block the hotspot region using the MLC. Figure 2 shows the beam's eye views (BEVs) of (a) one of the main beams and (b) the corresponding sub-beam. The positions of the MLC in the sub-beams were determined to block the hotspot region without a margin in the BEV. Because the hotspot region tends to be located in the frontal and occipital lobes at the edge of the irradiation field, the isocenter was not blocked in the sub-beams in almost all cases.

To identify the hotspot region, the 3D dose distribution of the original plan was extracted using the ESAPI. In the ESAPI, the 3D dose distribution was defined in the DICOM coordinate system (DCS) fixed in a patient. The hotspot region defined in the DCS was projected onto the BEV using the following three steps: (1) the coordinate transformation of translation from the DCS (x, y, z) to the coordinate system (X, Y, Z), where the isocenter was at the origin (isocenter coordinate system,

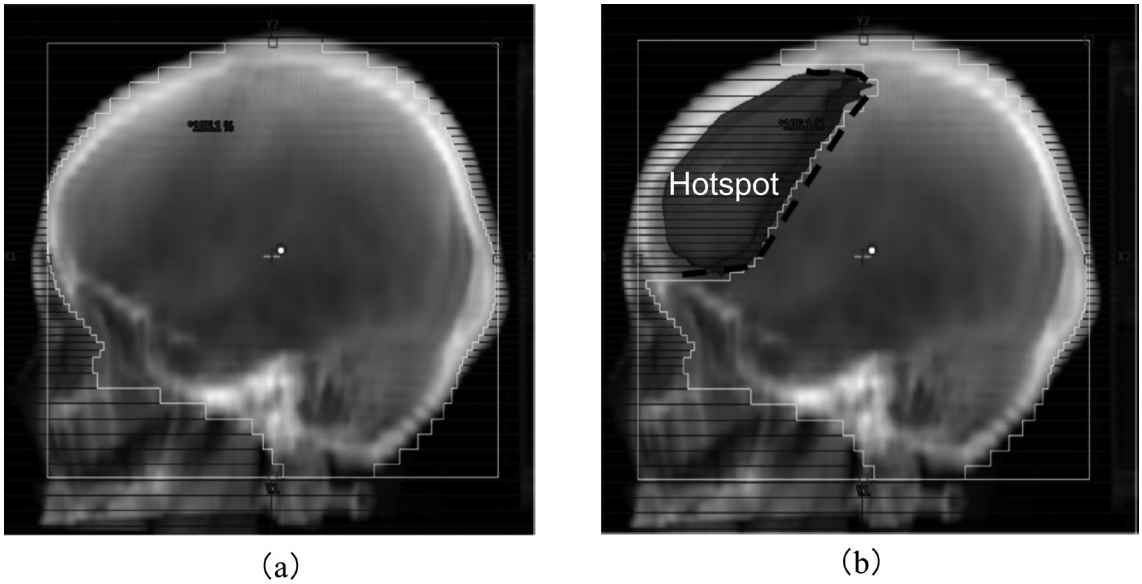

Figure 2 Beam's eye views with the multileaf collimator shapes of (a) the main beam and (b) sub-beam in the field-in-field plan. The projection of the hotspot region in the original plan is indicated by Hotspot in (b).

ICS), was performed.

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} -x_{\mathrm{iso}} \\ -y_{\mathrm{iso}} \\ -z_{\mathrm{iso}} \end{pmatrix} + \begin{pmatrix} x \\ y \\ z \end{pmatrix},$$

where $(x_{\mathrm{iso}}, y_{\mathrm{iso}}, z_{\mathrm{iso}})$ are the coordinates of the isocenter in the DCS. (2) The coordinate transformation from the ICS to a coordinate system (X', Y', Z') fixed at the gantry with the isocenter as the origin (beam coordinate system [BCS]) was performed.

$$\begin{pmatrix} X' \\ Y' \\ Z' \end{pmatrix} = \begin{pmatrix} \cos\theta_c & 0 & \sin\theta_c \\ 0 & 1 & 0 \\ -\sin\theta_c & 0 & \cos\theta_c \end{pmatrix} \begin{pmatrix} \cos\theta_g & \sin\theta_g & 0 \\ -\sin\theta_g & \cos\theta_g & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} X \\ Y \\ Z \end{pmatrix},$$

where θ_c and θ_g are the collimator and gantry angles, respectively. The senses of rotation of the collimator and gantry followed the IEC61217 scale convention, and the directions of the X', Y', and Z' axes are presented in Figure 3. (3) A point in the BCS was projected onto the BEV plane at the isocenter ($Y' = 0$ in the BCS) as follows:

$$\begin{pmatrix} X_{BEV} \\ 0 \\ Z_{BEV} \end{pmatrix} = \begin{pmatrix} \dfrac{\mathrm{SAD}}{(Y'+\mathrm{SAD})} & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & \dfrac{\mathrm{SAD}}{(Y'+\mathrm{SAD})} \end{pmatrix} \begin{pmatrix} X' \\ Y' \\ Z' \end{pmatrix},$$

where SAD is the source-to-isocenter distance of 100 cm. With the projection, point B' was moved to point B_{BEV}, as shown in Figure 3. By projecting all hotspots in the 3D dose distribution, the projection of the hotspot region on the BEV was obtained as a binary image (Figure 4).

The MLC leaves in the sub-beams were moved to cover the hotspot region in the BEV as in Figure 4. An MLC leave which is on the side of a hotspot was used to block the hotspot if the hotspot did not cross the vertical center line of a field. If a hotspot region crossed the vertical center line of a field, the horizontal distances from the center of the field to the outer edges of the hotspot were measured, as

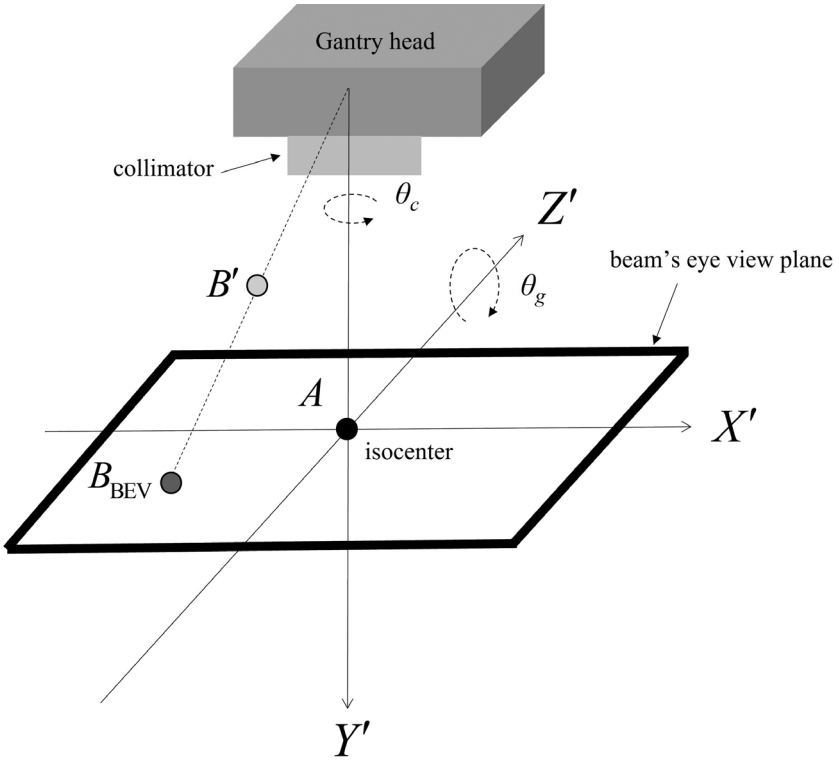

Figure 3 Definition of the beam coordinate system (BCS) and beam's eye view (BEV) plane, which are fixed to the collimator and rotate with the collimator and gantry. When both the collimator and gantry angles are 0°, the X' axis is in the cross-plane direction, Y' axis is in the vertical direction, Z' axis is in the in-plane direction, rotation of the collimator is around the Y' axis (θ_c), and rotation of the gantry is around the Z' axis (θ_g). Point A is the isocenter. By projecting onto the BEV plane, the point B' (X', Y', Z') is moved to B_{BEV} (X_{BEV}, 0, Z_{BEV}).

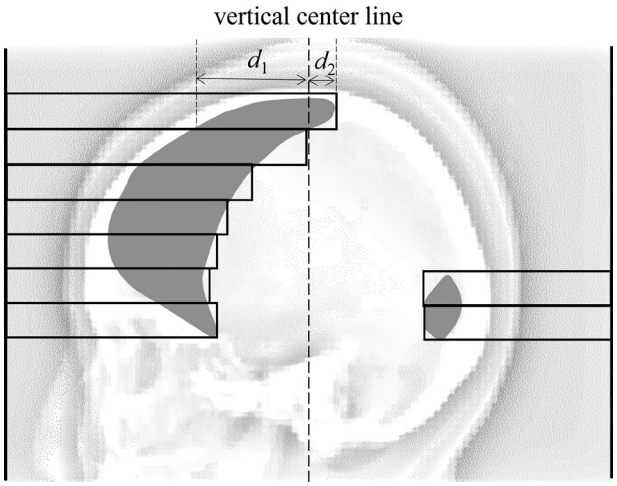

Figure 4 Schematic beam's eye view of a sub-beam. The gray regions indicate projected hotspots
The dotted line is the vertical center line of the field of the sub-beam. Rectangles indicate multileaf collimator leaves, which completely cover the hotspots. d_1 (d_2) is the horizontal distance from the vertical center line to the outer edges of the hotspot on the left (right) side.

d_1 and d_2 in Figure 4. The side of an MLC leave which had a longer horizontal distance (d_1 in Figure 4) than the other side was selected to block the hotspot. The MLC leaves were moved as far as completely covering the hotspot.

The weights of the main beams were reduced to decrease the dose in the hotspot regions as follows:

$$w_{\text{main}}^{i,\text{FIF}} = f w_{\text{main}}^{i},$$
$$f = \frac{D_{\text{th}}}{D_{\text{max}}}, \quad (1)$$

where D_{max} is the maximum dose in the original plan, D_{th} is the dose threshold for the hotspot regions, i is the index for the main beams (i =1, 2), and w_{main}^{i} and $w_{\text{main}}^{i,\text{FIF}}$ are the weights in the original and FIF plans for the main beams, respectively. The weights of the sub-beams ($w_{\text{sub}}^{i,\text{FIF}}$, i =1, 2) were assigned as follows:

$$w_{\text{sub}}^{i,\text{FIF}} = (1-f) w_{\text{main}}^{i}. \quad (2)$$

Approximately, with the new weights, w_{main}^{i} and $w_{\text{sub}}^{i,\text{FIF}}$, the doses in the unblocked regions do not change and those in the blocked regions reduced by f.

With the new MLC shapes for the sub-beams and new weights for the main beams and sub-beams, the 3D dose distribution for the FIF plan with the two sub-beams (FIF) was calculated in the TPS and dose indices were evaluated. In this study, D_{th} was fixed at 105% of the prescribed dose at the isocenter.

FIF plan in Step 2

After Step 1, when the dose coverage of the target is greatly reduced, there is a possibility that the reduction in the dose coverage can be alleviated by increasing the number of sub-beams. This is because an increase in the number of sub-beams provides higher degrees of freedom for dose modification. In this study, when the reduction in $D_{95\%}$ of the PTV was >1% in Step 1, FIF treatment planning with four sub-beams was performed. The criterion of 1% reduction was arbitrarily adopted as an example. It can be changed to a different criterion easily.

In this step, the sub-beams were added sequentially. First, two sub-beams were added to the original plan, as in Step 1. The high-hotspot regions that received a dose greater than the intermediate threshold $D_{\text{ith}}=(D_{\text{max}}+D_{\text{th}})/2$ were identified in the dose distribution in the original plan, as shown in Figure 5a. The same procedures as in Step 1 were performed by replacing D_{th} with D_{ith}. After the procedures, the FIF plan with the two sub-beams was obtained. Second, two sub-beams were newly added. The low-hotspot regions that received a higher dose than D_{th} were identified in the FIF plan with the two sub-beams. The MLC apertures of the two extra sub-beams were shaped to block the low-hotspot regions in the BEV, as shown in Figure 5b.

The weights for the main beams and the first two sub-beams were assigned, as shown in Eqs. (1) and (2), where D_{th} is replaced by D_{ith}. The weights for the main beams $w_{\text{main}}^{i,\text{FIF2nd}}$ and extra sub-beams $w_{\text{sub}}^{i,\text{FIF2nd}}$ in the four sub-beams FIF plan were assigned as follows:

$$w_{\text{main}}^{i,\text{FIF2nd}} = f_{\text{2nd}} w_{\text{main}}^{i,\text{FIF}},$$
$$w_{\text{sub}}^{i,\text{FIF2nd}} = (1-f_{\text{2nd}}) w_{\text{main}}^{i,\text{FIF}},$$
$$f_{\text{2nd}} = \frac{D_{th}}{D_{\text{ith}}^{\text{max}}},$$

where i is the index for the main beams (i =1, 2), and $w_{\text{ith}}^{\text{max}}$ is the maximum dose in the FIF plan with the two sub-beams. The 3D dose distribution was calculated for the FIF plan with four sub-beams (FIF-4SF), and the dose indices were evaluated.

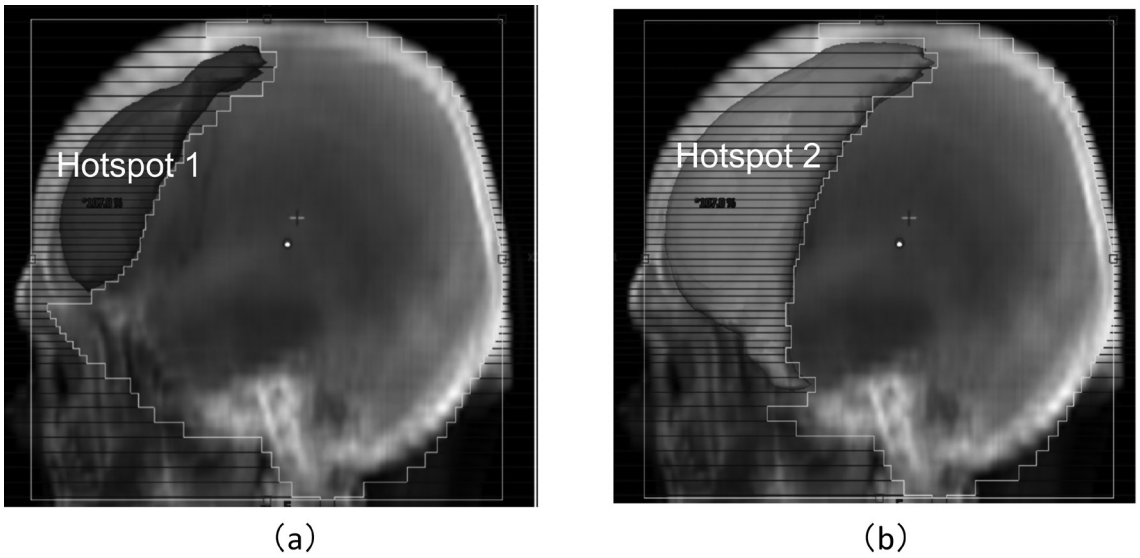

Figure 5 Beam's eye views (BEVs) with the multileaf collimator shapes of the sub-beams in the four-sub-beam field-in-field plan. (a) The BEV of one of the first sub-beams. The high-hotspot region of 106.4 % of the prescribed dose in the original plan is represented by Hotspot 1. (b) The BEV of one of the second sub-beams. The low-hotspot region of 105 % of the prescribed dose after the first step is indicated as Hotspot 2.

Application of the automated FIF algorithm

Patients

We used the planning data of 22 patients with brain metastases from lung, breast, and rectal cancers treated with WBI from January 2016 to December 2017 at Showa University Yokohama Northern Hospital. The study protocol was approved by the institutional review board (##17HO92) of our hospital, and the need for informed consent was waived. This study was conducted in accordance with the Declaration of Helsinki and Japanese ethical guidelines for epidemiologic research.

The clinical treatment plans were used as the original plan for the automated FIF algorithm. All plans used two laterally opposing fields with 10 MV of X-ray energy and prescribed a total dose of 30 Gy (2 Gy per fraction) or 31.2 Gy (1.2 Gy per fraction delivered twice daily) at the isocenter. The dose calculation algorithm used for all plans was an analytical anisotropic algorithm[15] in the TPS (Eclipse, version 13.7.14; Varian Medical Systems, USA). The PTV included the cerebral parenchyma, with a margin of 2 or 3 mm. The original plans were created for a linac (TrueBeam STx; Varian Medical Systems, USA) with the MLC containing 14 pairs of leaves with a 2.5-mm width and 32 pairs of leaves with a 5-mm width. The dose grid size was 2.5×2.5×2.5 mm^3 in the TPS.

Creation of a manual FIF plan for comparison with automatic FIF

We also manually created FIF plans from the original plans by a board-certified physicist . Two sub-beams were created by copying the two main beams. Hotspot regions in the original plan were blocked using MLC leaves in both sides of the sub-beams. The hotspot regions were visually identified in the BEV. The same threshold for hotspot was taken as in the automatic FIF case. The same weights were given to the sub-beams to remove the hotspot. The procedures of making subfields and adjusting beam weights are almost the same as for the automatic FIF case.

Dose index evaluation

$V_{105\%}$, $V_{95\%}$, $D_{95\%}$, D_{max}, and the homogeneity index (HI) of the PTV in the semiautomatic FIF plans were compared with those in the original and manual FIF plans. $V_{105\%}$ was used as an index to indicate whether the hotspot decreased using the FIF technique. $V_{95\%}$ and $D_{95\%}$ were used as indicators of dose coverage. D_{max} was used as an indicator of excess dose. The dose and volume were expressed as the relative dose to the prescribed dose and relative volume to the PTV, respectively. D_V was the dose to a specified fractional volume V in the PTV. V_D was the volume that received at least the dose D in the PTV. The HI is defined as

follows:

$$\mathrm{HI} = \frac{D_{2\%} - D_{98\%}}{D_{50\%}},$$

by normalizing the difference between $D_{2\%}$ and $D_{98\%}$ with $D_{50\%}$[16].

$V_{95\%}$, $D_{95\%}$, and $D_{\max}$ of the semiautomatic FIF and original plans and those of the semiautomatic FIF and manual FIF plans were statistically compared. Since these data did not follow a normal distribution, the Wilcoxon signed-rank test was performed using the JMP software (version 12.2.0; SAS Institute Inc., USA). A P-value of <0.05 was accepted as significant.

Measurement of the processing time for creating the sub-beams and adjusting the weights

The manual FIF creation time was measured with a stopwatch from the start of FIF creation to the end of the dose calculation. The elapsed time between the opening of the original plan in the TPS and the final dose calculation was measured in the automatic FIF script. The processing time included the time for creating sub-beams, adjusting the beam weights, and dose calculation.

Results

Comparison of dose indices and processing time

A total of 21 cases resulted in FIF and one case in FIF-4SF. Figure 6a-e shows the box plots of $V_{105\%}$, $V_{95\%}$, $D_{95\%}$, $D_{\max}$, and HI in the original, manual FIF, and semiautomatic FIF plans. The creation

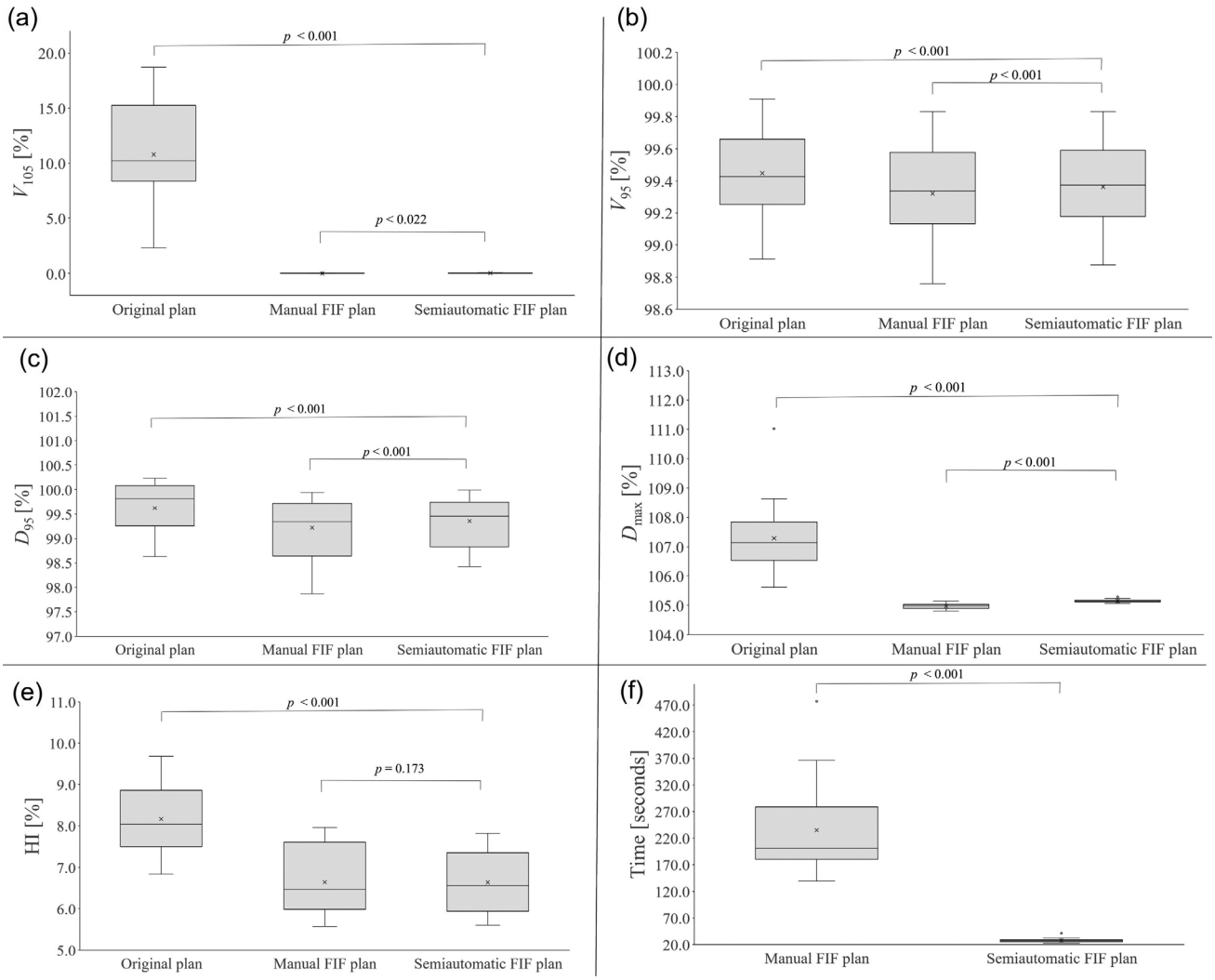

Figure 6 The box plots for (a) $V_{105\%}$, (b) $V_{95\%}$, (c) $D_{95\%}$, (d) $D_{\max}$, and (e) HI in the original, manual field-in-field (FIF), and semiautomatic FIF plans. The box plot for the processing time in the manual FIF and semiautomatic FIF plans is also presented in (f). p indicates P-values for the Wilcoxon signed-rank test.

times for the FIF plans are presented in Figure 6f. In manual FIF and semiautomatic FIF plans, $V_{105\%}$ decreased by almost 100% compared with the original plans, as shown in Figure 6a. This indicates that the hotspot region above D_{th} almost disappeared when using the semiautomatic FIF technique. $V_{95\%}$ values on average (± one standard deviation) for the original, manual FIF, and semiautomatic FIF plans were 99.4 ± 0.3%, 99.3 ± 0.3%, and 99.4 ± 0.3%, respectively (Figure 6b). Similarly, $D_{95\%}$ values on average for the original, manual FIF, and semiautomatic FIF plans were 99.6 ± 0.5%, 99.2 ± 0.6%, and 99.4 ± 0.5%, respectively (Figure 6c). D_{max} values on average for the original, manual FIF, and semiautomatic FIF plans were 107.3 ± 1.1%, 105.0 ± 0.1%, and 105.1 ± 0.1%, respectively (Figure 6d). The HIs values on average for the original, manual FIF, and semiautomatic FIF plans were 8.2 ± 0.8%, 6.6 ± 0.8%, and 6.6 ± 0.7%, respectively (Figure 6e).

The paired Wilcoxon signed-rank test indicated that there were significant differences in $V_{105\%}$, $V_{95\%}$, $D_{95\%}$, and D_{max} in the original and manual FIF plans compared with those in the semiautomatic FIF plans. There was also significant difference in HI in the original plans compared with that in the semiautomatic FIF plans.

The semiautomatic FIF planning times, which were defined as the processing times by the automatic FIF script to generate the sub-beams and dose calculations, were 25–29 s for FIF (Step 1) and 41 s for FIF-4SF (Step 2). The average time including FIF and FIF-4SF was 28 ± 4 s. The processing time for the semiautomatic FIF plan technique significantly decreased by 207 ± 84 s compared with that for the manual FIF plans (Figure 6f).

Comparison of dose distribution

Figure 7 shows a typical example of the dose distributions in the (a) original, (b) manual FIF, and (c) semiautomatic FIF plans for the case in

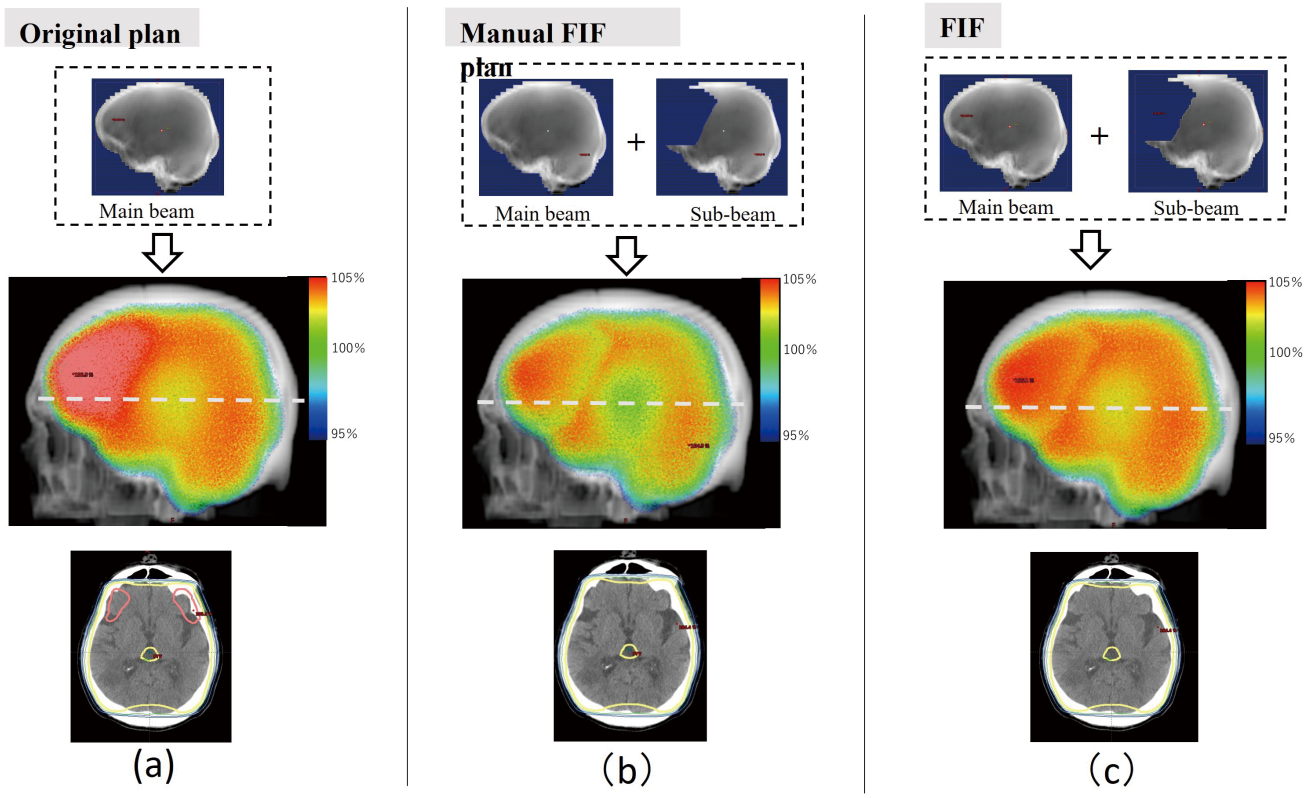

Figure 7 Dose distributions of the original, manual field-in-field (FIF), and semiautomatic FIF plans for case no. 22. (a) Field shape and dose distributions of the original plan. The upper, middle, and lower panels show the field shape, 3D dose distribution in the beam's eye view, and 2D dose distribution in a slice, respectively. The slice position is shown in the middle panel with a white dotted line. (b) Same as (a) but for the manual FIF plan. (c) Same as (a) but for the FIF plan in Step 1 (FIF). In the 2D dose distributions, the yellow lines are the 100% isodose lines, and the pink lines are the 105% isodose lines. The doses are relative to the prescribed dose.

Step 1. As shown by the dose distributions in the axial plane in Figure 7a and 6c, the 100% isodose line did not change in Step 1 even after the hotspot region exceeding 105% of the prescription dose disappeared using the semiautomatic FIF technique. The dose distributions for the manual and FIF plans were almost the same, as indicated in Figure 7b and c. The dose volume histograms (DVHs) of the PTV of the original, manual FIF, and FIF plans are shown in Figure 8a. D_{max} remarkably decreased in the manual FIF and FIF plans, while there was almost no change in $D_{95\%}$.

One case was categorized into Step 2 in this study. Figure 9 shows the dose distributions in the

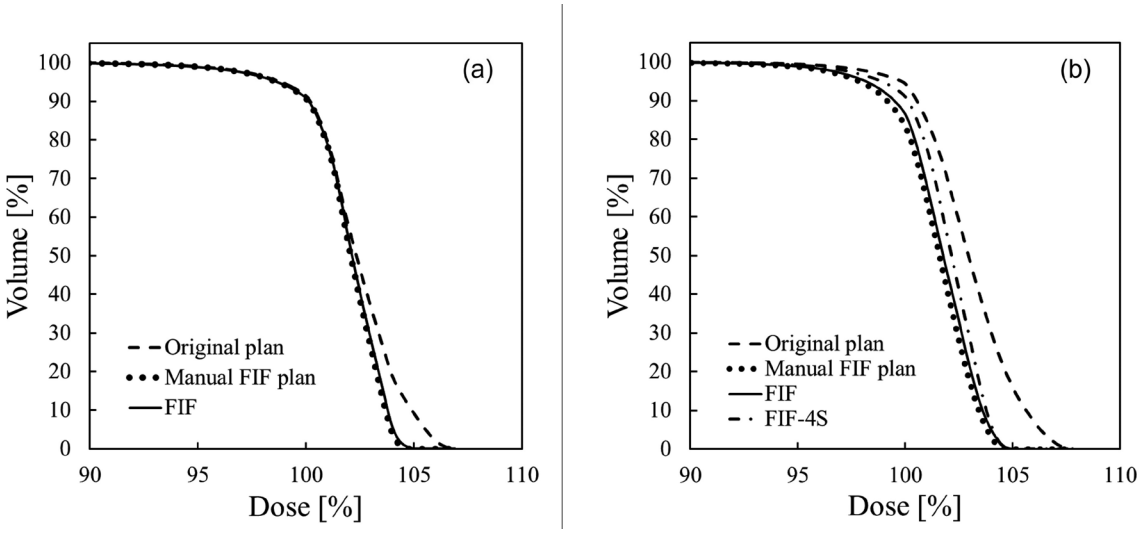

Figure 8 Dose volume histograms (DVHs) of planning target volume (PTV) in the original, manual field-in-field (FIF), and semiautomatic FIF plans. (a) DVHs of PTV in the original (dashed line), manual FIF (dotted line), and two-sub-beam FIF plans in Step 1 (solid line). (b) DVHs of the PTV in the original (dashed line), manual FIF (dotted line), FIF (solid line), and four-sub-beam FIF plans in Step 2 (FIF-4SF, dashed-dotted line).

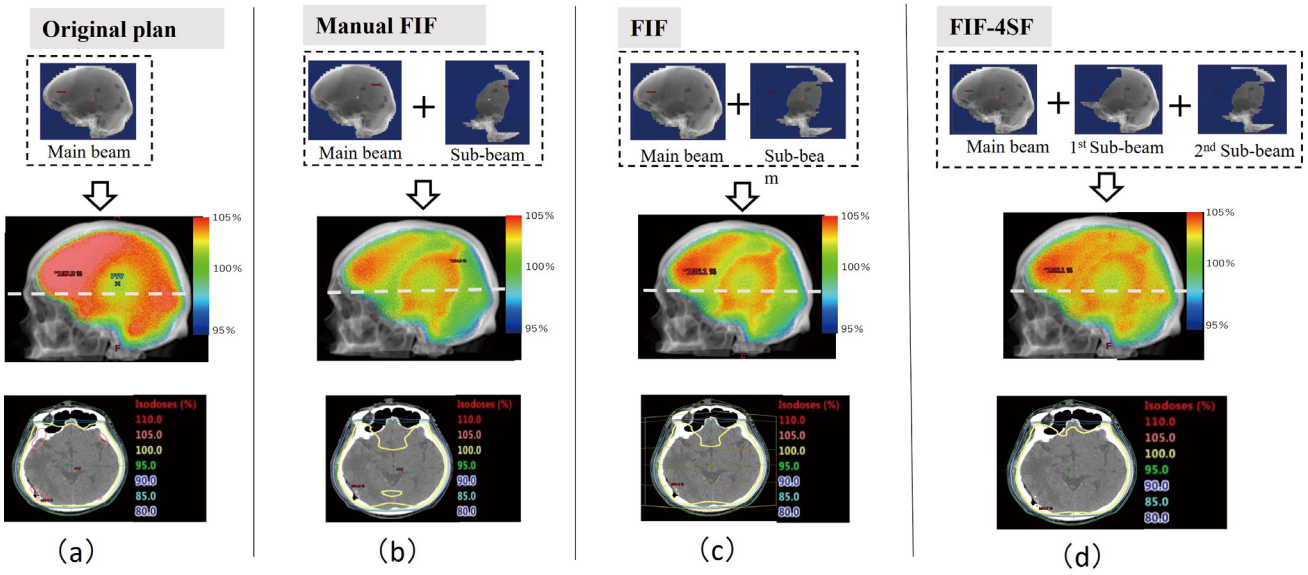

Figure 9 Dose distributions of the original, manual field-in-field (FIF), and semiautomatic FIF plans with the different numbers of sub-beams (FIF and FIF-4SF). (a) Field shape and dose distributions of the original plan. The upper, middle, and lower panels show the field shape, 3D dose distribution in the beam's eye view, and 2D dose distribution in a slice, respectively. The slice position is indicated in the middle panel with a white dashed line. (b) Same as (a) but it is for the manual FIF plan. (c) Same as (a) but it is for the two-sub-beam FIF plan in the Step 1 (FIF). (d) Same as (a) but it is for the four-sub-beam FIF plan in the Step 2 (FIF-4SF). In the 2D dose distributions, the yellow lines are the 100% isodose lines, and the pink lines are the 105% isodose lines. The doses are relative to the prescribed dose.

(a) original, (b) manual FIF, (c) FIF, and (d) FIF-4SF plans for this case. The FIF plan was rejected because the reduction in $D_{95\%}$ was >1% (1.6%), and Step 2 was performed. Figure 9d shows the dose distribution in the FIF-4SF plan. In the dose distributions of the manual FIF and FIF plans (Figure 9b and c), a low-dose region appeared in the field edge of the sub-beam. As shown in the axial image in Figure 9c, the 100% dose region in the FIF plan was smaller than that in the original plan. In the dose distribution in FIF-4SF (Figure 9d), the dose reduction around the field edge of the sub-beam was small compared with the dose distribution in the FIF plan.

Comparing the DVHs of the manual FIF, FIF, and FIF-4SF plans in Figure 8b, the maximum dose region that corresponds to the tail part of the DVHs is nearly similar in the three FIF plans. By increasing the number of sub-beams from two to four in the FIF-4SF plan, $D_{95\%}$ became close to the corresponding values in the original plan. The reduction of $D_{95\%}$ from the original plan was 1.6% in the FIF plan, and 0.7% in the FIF-4SF plan.

Discussion

In this study, sub-beam creation and weight adjustment in FIF treatment planning were automated. These are the time-consuming parts in the FIF treatment planning and require ≥6 min if performed manually, as shown in Figure 6. The automatic script can be started with one click and requires <2 min, and reduces the burden on the treatment planner. Comparing the semiautomatic FIF and manual FIF plans, there was no significant difference in the HI; there were significant differences in $V_{95\%}$ and $D_{95\%}$. However, the differences of $V_{95\%}$ and $D_{95\%}$ were small. Thus, we found that the semiautomatic planning script could create the FIF plans almost equivalent to the manual FIF plans.

If a TPS has a scriptable interface, the proposed method can be easily introduced into the TPS. In this study, we adopted the change in $D_{95\%}$ as the criterion for selecting the FIF technique. The criterion can be easily modified by adjusting the parameters in the automatic FIF planning script, depending on the treatment site and protocol of each institution. The threshold value for the hotspot regions and number of sub-beams can also be changed. For example, if D_{th} is lowered, both D_{max} and $D_{95\%}$ will decrease. If the reduction of $D_{95\%}$ is acceptable, lowered D_{th} can be used. In this study, D_{th} was taken as 105% of the prescribed dose to make the maximum dose less than 107% of the prescribed dose considering the ICRU recommendation. D_{th} can be changed by considering a balance between D_{max} and $D_{95\%}$. In this study, the beam energies of the sub-beams were the same as those of the main beams (10 MV). The use of different energies for the sub-beams can be easily implemented and may improve the dose distribution.

Semiautomatic FIF planning was performed for the WBI cases, which were previously treated with the two-lateral-opposing-field technique. In the treatment plans with two-lateral-opposing technique, hotspot regions appeared in the frontal and occipital lobes, which are laterally thin parts of the brain[2, 4]. Although there were significant differences in $V_{95\%}$ and $D_{95\%}$ between the original and semiautomatic FIF treatment plans, the average decreases in $V_{95\%}$ and $D_{95\%}$ were 0.1% and 0.4%, respectively. The effect of the reductions in $V_{95\%}$ and $D_{95\%}$ is expected to cause minimal deterioration of the quality of the semiautomatic FIF plans.

In this study, we adopted a 2-Step schema to select FIF technique. In the two-step FIF scheme, the number of sub-beams was increased from two to four to improve dose coverage of PTV, when the reduction in $D_{95\%}$ was >1%. One case resulted in the FIF-4SF plan. The reduction of $D_{95\%}$ compared with those in the original plan were 1.6% in the FIF plan. In the FIF-4SF plan, the reduction became 0.7% by increasing sub-beams. This indicates that the two-step FIF scheme can individualize the complexity of a treatment plan (beam number) depending on a patient.

The monitor unit (MU) for sub-beams becomes small by increasing the number of sub-beams. In the FIF plans, the weight of the main beam and sub-beam on the average was 0.979 ± 0.010 and 0.021 ± 0.010, respectively. In the manual FIF plans, the weight of the main beam and sub-beam on the average was 0.975 ± 0.011 and 0.036 ± 0.046. In the FIF-4SF plan, the weights of the main beams, first sub-beams, and second sub-beams were 0.973, 0.013, and 0.014, respectively. Small MU may cause dose uncertainty. To avoid the dose uncertainty due to small MU, the algorithm to limit the minimum MU is easily implemented in the automatic FIF

script.

The average processing times were 27 s and 41 s for the FIF and FIF-4SF plans, respectively.

A longer time was required to create the FIF-4SF plan than the FIF plan. The average time of the 3 D dose calculation for the FIF plans was 19 s and the calculation time of the 3D dose calculation for the FIF-4SF plan was 28 s. The difference in the processing time was mainly explained by the time for the 3D dose calculation.

We used WBI cases to demonstrate the feasibility of the proposed method. Because the proposed method only requires a conventional plan without sub-beams as the input, it can be easily applied to other treatment sites, such as the whole breast[12] and esophagus.

Kim et al. reported on FIF planning automation for whole-breast irradiation[12]. They used the ESAPI in their automatic technique. In their method, information of the original plan was exported to an executable program outside the TPS in the DICOM and DICOM-RT formats. In their method, the MLC shapes and beam weights for the sub-beams were calculated and automatically imported into the TPS. Using their method, FIF plans with the same quality as those manually created were obtained. We used almost the same approach as that of Kim et al., but our procedures can be performed within the TPS. Furthermore, we implemented several steps to personalize the treatment plan to the patient.

Yu et al. implemented an automated MLC shaping technique for WBI using deep learning. They could produce dose distribution almost equivalent to manually produced treatment plans. They used the two-opposing-lateral-field technique and obtained relatively high maximum dose of approximately 110%. By combining their technique with ours, a fully automated treatment plan for WBI can be realized.

By automating the sub-beam shape and adjusting the weight, these processes can be standardized[17]. The manual shaping of sub-beams and adjustment of beam weights depend on the skill and preference of the treatment planners. The automation of FIF planning can reduce the variability originated from the skill and preference of the treatment planners and the possibility of human error. However, because the transition from manual to automated planning can potentially lead to systematic errors that are difficult to detect[18], the final verification must be performed by humans.

A limitation of the present study is all manual FIF plans were created by one physicist. The processing time and the quality of the FIF plans probably depend on the experience of treatment planners. In this study, the similar plans were obtained for the manual and automatic FIF planning. One reason of this similarity is probably that a single person made the manual FIF plans. The manual plans would diverge from the automatic FIF plans if multiple persons created the manual plans. However, the indication that the automatic FIF creation reduces the burden of the treatment planners is still valid because the sub-beam creation and weight adjustment are automatically performed.

We developed a semiautomatic FIF planning method and implemented it in a TPS. By applying it to WBI, we confirmed that the semiautomatic FIF technique could reduce hotspot regions with a slight change in the PTV coverage compared with the original plan. When combined with a selection of an FIF scheme individualized to each patient, its performance was equal to or better than the manual FIF plan.

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Author contributions

HW, SS, TK, HN, TI, CK, KU, JT and KS participated in the study design and data interpretation. Satoru Sugimoto performed manual treatment planning. KK participated in treatment planning data collection. HW wrote the draft manuscript. SS, TI, JT and KS reviewed and revised the draft and final manuscript. All authors read and approved the final manuscript.

Conflicts of interest statement

There are no conflicts of interest in relation to this study.

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Reviews

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Sports and Kinetic Visual Acuity

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Kinetic vision acuity (KVA) is an index developed in Japan that refers to the capacity to recognize a moving object that moves back and forth against the observer. This review outlines the history of KVA and studies on KVA conducted at the Faculty of Health and Sports Science of Juntendo University, i.e. characteristics of KVA in athletes, factors associated with KVA, sports and age-dependent decline of KVA, and effects of docosahexaenoic acid (DHA) and astaxanthin on KVA. KVA was defined in the early 1960s, and the measurement device was invented in 1968. Studies at the Faculty of Health and Sports Science began in the 1990s. In track-and-field athletics and skeleton, a winter downhill event, higher-ranked athletes had higher KVA than lower-ranked athletes. Although KVA cannot be predicted from static visual acuity or reaction time, a significant correlation was found between KVA and the peak latency of visual-evoked potentials. KVA could not be improved by training and did not change between age of 8 and 17 years. In contrast, habitual practice in kendo may inhibit the age-dependent decline in KVA. DHA may also improve KVA in subjects with low KVA; however, astaxanthin did not improve KVA.

Key words: kinetic vision acuity, dynamic visual acuity, athletics

Introduction

The ability to recognize a moving object differs from that to recognize a stationary object. Kinetic vision acuity (KVA) is an index developed in Japan to measure the ability to recognize moving objects that move back and forth against the observer. Since the 1990s, studies on the relationship between KVA and sports have been conducted at Juntendo University Faculty of Health and Sports Science. However, the overall results remain obscure because they were published in various journals mostly in Japanese. Therefore, this review outlines the history of KVA developed in Japan and studies conducted at the Faculty of Health and Sports Science of Juntendo University, i.e. characteristics of KVA in athletes, factors associated with KVA, sports and age-dependent decline of KVA, and effects of docosahexaenoic acid (DHA) and astaxanthin on KVA.

Brief history of KVA

The representative capacity to recognize moving objects includes dynamic visual acuity (DVA) and KVA. DVA is the ability to recognize objects moving in all directions, including left, right, up, and down when the object, the observer, or both, are moving, at an equal distance from the observer. In 1949, Ludvigh showed that stationary objects are more clearly visible than moving ones[1)]. Moreover, in 1953, he reported that the DVA decreased as the angular velocity of the test object moving in

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the horizontal plane increased from 10 to 170 deg/sec. There was also a significant difference in DVA in subjects with almost the same static visual acuity (SVA)[2]. A recent review summarizes the characteristics of DVA in comparison with SVA[3]. In Japan, Kowa (Nagoya, Japan) launched the first DVA-measuring device HI-10 in 1998. The device had been mainly used under the guidance of the Japan Sports Vision Association although it is no longer manufactured and sold.

Hagino et al. of Nagoya University began vigorous research on the ability to recognize moving objects in the 1950s[4]. Suzumura defined KVA as the ability to recognize an object moving back and forth in the distance against the observer[4, 5]. Suzumura invented a device to measure KVA in 1968[6]. The device, AS-4A, was launched by Kowa (Nagoya, Japan) in 1966 and has been mainly used in Japan as a tool for driving education for automobile drivers. Currently, the AS-4Fα (Figure 1), the successor to the AS-4A (Kowa, Nagoya, Japan), is available, which identifies KVA as the ability to recognize the Landolt ring approaching the observer from 50 m from the front at 30 km/h (Figure 2).

To recognize a moving object accurately, it is necessary to quickly catch the object near the central fossa, adjust it, and bring it into focus. Moving objects are usually more difficult to see clearly than stationary ones; therefore, KVA does not exceed SVA. Suzumura has clarified that (1) KVA decreases as the speed of the target increases, (2) individual variability in KVA is large, (3) decline in KVA cannot be perceived, and (4) decline in KVA is unrelated to SVA[7].

Figure 1 Kinetic visual acuity tester AS-4Fα (Kowa, Nagoya, Japan)

DVA is widely used in Europe and the United States, whereas KVA is mainly used in Japan. In PubMed, search results for "dynamic visual acuity" included 349 papers, 266 of which were published in 2001 or later. Meanwhile, search results for "kinetic visual acuity" included 31 papers, only 5 of which were published in 2001 or later (searched on February 2, 2022). The large difference in the number of papers can be attributed to KVA being developed in Japan and many previously published studies being in Japanese.

KVA of athletes

In 1974, Sanderson et al. reported a significant

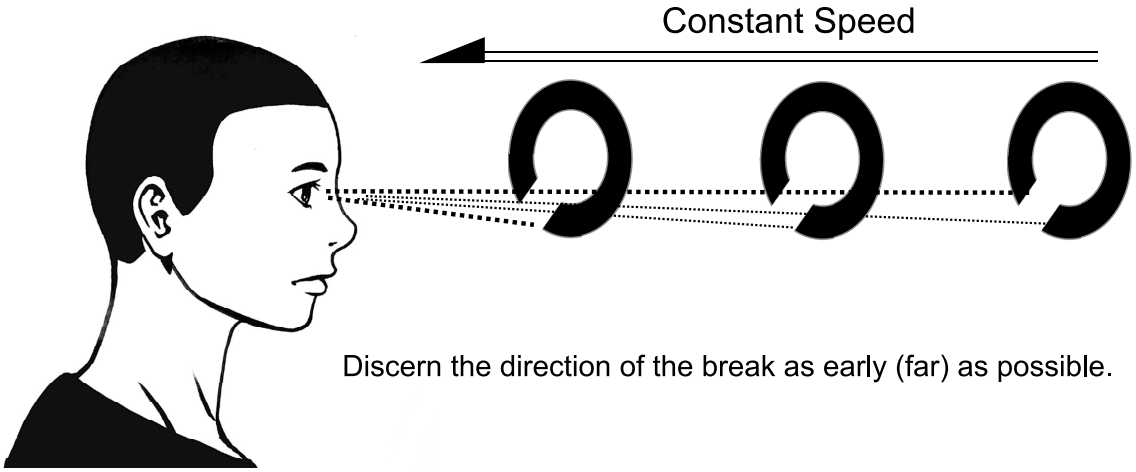

Figure 2 Schema to measure kinetic visual acuity. A Landolt ring is approaching from front at a constant speed. The observer discerns the direction of the break as early (far) as possible. This ability is measured as kinetic visual acuity.

correlation between DVA and ball-catching performance and an absence of significant correlation between DVA and SVA[8]. In healthy young adults, those who engage in sports activities were found to have better DVA than those who do not[9].

In Japan, the relationship between KVA and the batting ability of baseball players was reported at the annual meeting of the Japanese Society of Physical Education in 1971[10]. However, there were no reports on the relationship of KVA with sports for the next 20 years. In 1992, Ishigaki et al. reported that in top-level Japanese basketball, volleyball, soccer, and baseball players, athletes with a higher competitive ability also had higher KVA[11]. In 1995, people who played tennis continuously for more than 7 years were reported to have better KVA than those with no sports history[12]. In addition, at the 1996 annual meeting Japan Society of Physical Education, Health and Sport Sciences, a professional baseball team case study reported that KVA was higher in regular players than in second-unit players when they joined the team[13].

Studies on KVA at Juntendo University's School of Sports and Health Science began in 1996 with the DHA study by Sawaki & Yoshigi et al. described in "Effects of DHA and astaxanthin on KVA" below. Sakuma et al. investigated the SVA and KVA of male college track-and-field athletes and found that both SVA and KVA were higher in athletes engaged in steeplechase, jump, and mixed events than in short-, middle-, and long-distance runners and throwers and that SVA and KVA are higher in athletes with higher competitive ability[14]. Aoki et al. investigated elite male pole vaulters and long jumpers and reported that there was no difference in KVA between pole vaulters and long jumpers and that the top competitive pole vaulters had higher SVA and KVA than the bottom pole vaulters[15]. In addition, Inoue et al. compared the skeleton athletes competing in international sledding competitions and lower group competing in the Japanese National Championships to find that KVA was significantly higher in the upper group than in the lower group, whereas no difference was seen in SVA[16].

Factors associated with KVA

Sado et al. examined the SVA and KVA of freshmen of the Faculty of Health and Sports Health Science whose SVA was 1.0 or higher[17]. The number of subjects was 92 (78 men and 14 women aged between 18 and 20 years). SVA was greater than 1.0, whereas KVA ranged from 0.12 to 1.30. KVA could not be estimated from SVA although there was a weak but significant positive correlation between SVA and KVA ($r = 0.486$ in the right eye, $r = 0.490$ in the left eye, and $r = 0.384$ in both eyes)[17].

Kohmura et al. determined the association between KVA and reaction time. The reaction time, often used in sports science, reflects the ability to identify and respond quickly to visual cues. However, the correlation between KVA and reaction time was not apparent[18, 19]. Yoshigi et al. also analyzed the relationship between KVA and each component of visual-evoked potential and found a significant correlation between KVA and the peak latency of visual-evoked potentials; the better the KVA, the shorter the peak latency[20].

Studies have been reported on the effects of training on KVA in baseball players. Kohmura et al. conducted an 8-week training experiment wherein college baseball players were trained to look at the ball at the batter's box or to use commercially available software for visual function training. KVA was not improved by either training although some effects on visual function were observed[21]. A subsequent study with junior high school students also revealed that training did not improve KVA[22].

Kohmura et al. studied 867 men and women aged 8–17 years and reported that DVA developed gradually with age, but KVA did not change from age 8 to 17 years[23]. Therefore, KVA is expected to reach almost the same level as adults by the age of 8 years[23].

As described above, KVA has a large individual variation, may not be improved by training, and matures up to 8 years old, KVA could be available for finding talent in the junior period in sports.

Sports and age-related decline of KVA

Nakamura et al. compared young and elderly kendo players ($n = 30$) with age-matched nonathletes ($n = 30$) and found that KVA significantly decreased with age, whereas contrast sensitivity, eye movement, and instantaneous visual acuity were not affected by age or exercise habits[24]. At the same time, kendo players had a higher KVA than nonathletes ($p < 0.01$) when comparing ten young subjects each[24]. Age-related decline in

KVA has also been observed in young and middle-aged soccer players (n = 44) and in nonathletes of the same age group (n = 45)[25]. Recently, Kudo et al. reported kendo players (n = 41; 35.4 ± 15.7 years, range 19–65 years) had significantly higher KVA ($p < 0.01$) than nonexercisers (n = 65; 38.1 ± 17.1 years, range 19–71 years)[26].

Therefore, KVA declines with age, and training habits to gaze at moving objects, such as kendo, may suppress the age-related decline. However, the effect of sports remains unclear.

Effects of DHA and astaxanthin on KVA

DHA is an n-3 fatty acid found in the retina and the brain's gray matter[27]. In 1993, artificial milk containing fish oil was reported to improve visual acuity in preterm infants up to the fourth month of life[28]. This research led to the study of the effect of DHA on KVA.

In 1997, Sawaki et al. reported a randomized, double-blind study to examine the effects of DHA (1,500 mg/d) for 35 consecutive days in 44 male collegiate athletes and baseball players with an SVA of 1.0 or higher[29]. DHA intake significantly increased KVA from 0.87 ± 0.24 to 0.97 ± 0.21 ($p < 0.01$) but did not change SVA. The improvement was greater in participants with low KVA before intervention. No change was observed in the control group (equal amounts of soybean oil)[29].

To confirm the results of Sawaki et al.[29], Ishigaki et al. conducted a double-blind study of DHA (1,500 mg/d) for 30 consecutive days in 20 university long-distance runners and 12 fencing players[30]. The mean SVA significantly increased from 1.21 to 1.32 in the DHA group ($p < 0.05$), whereas there was no significant change in the placebo (safflower oil) group. The mean KVA improved from 0.65 to 0.69 in the DHA group, but not significant[30].

Subsequently, another study was conducted with 55 college athletes as participants: 8 tennis players, 18 volleyball players, 15 track-and-field athletes, and 14 baseball players (47 men and 8 women)[31]. A total of 28 participants did not take orthoptics, whereas 25 and 2 of them used contact lenses and glasses, respectively. DHA (1,500 mg/d) was given for 35 consecutive days, and their visual acuity was measured before and after the intervention. There were no significant differences in SVA and KVA between the groups (25 and 26 in the DHA and control groups, respectively). Low-contrast visual acuity improved in the DHA group but not in the control group ($p < 0.05$). When preintervention KVA was stratified by LogMAR = 0.3 (decimal acuity 0.5), subjects with lower preintervention KVA improved in the DHA group (n = 10) but not in the control group (n = 13; between-group comparison, $p < 0.05$). Therefore, DHA may improve KVA in subjects with low KVA[31].

Sawaki et al. also determined the effect of astaxanthin. A total of 18 male collegiate handball players ingested astaxanthin (6 mg/d, n = 9) or placebo (n = 9) for 4 weeks. No significant improvement was observed on SVA, KVA, or KVA/SVA[32].

Summary

This review aimed to outline the history of KVA followed by the studies on KVA conducted at Juntendo University Faculty of Health and Sports Science. Suzumura defined KVA as the ability to recognize an object moving back and forth in the distance against the observer, and invented a device to measure KVA. Studies on KVA has been conducted at Juntendo University Faculty of Health and Sports Science since 1990s. Athletes who require great attention to moving objects have a higher KVA. Further, athletes at higher levels of competition have higher KVA. The relationship of KVA to simple reaction time is unclear. In contrast, some studies suggest a correlation between KVA and peak latency of visual-evoked potentials. However, the mechanism to determine KVA requires more investigations. Alternatively, KVA represents a potential tool for discovering junior sports talent as it cannot be improved by training after 8 years old. Moreover, KVA declines with age although habitual exercise that requires a clear, quick vision of a moving object may suppress the age-related decline of KVA. In addition, DHA may improve KVA in subjects with low KVA. As described above, KVA is a unique visual acuity distinct from SVA. However, further studies are necessary to determine the practical application of KVA.

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Author contributions

K.S. conceptualized this review. Y.K., K.A., and Y.S. searched and screened literatures to review.

Y.K., K.A., M.N., S.M., and Y.S. summarized the literatures. Y.K. and Y.S. wrote the draft manuscript. K.S., Y.K., and Y.S. reviewed and edit the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Case Reports

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Histopathologic Features of Immune-related Adverse Events in the Gastrointestinal Tract: A Case of Severe Acute Respiratory Syndrome Coronavirus 2 and Cytomegalovirus Infection in a Patient with Lung Squamous Cell Carcinoma Receiving Immune Checkpoint Inhibitors

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In this article, we report the case of a patient with unresectable stage III squamous cell lung carcinoma who developed immune-related adverse events in the gastrointestinal tract following the administration of immune checkpoint inhibitors. The patient developed severe acute respiratory syndrome coronavirus 2 pneumonia and cytomegalovirus gastritis during immunosuppressive therapy for an immune-related adverse event. Cytomegalovirus infection was managed with the administration of ganciclovir.

Key words: immunotherapy, adverse events, colitis, gastritis, cytomegalovirus

Introduction

Immune checkpoint inhibitors are commonly used for the treatment of advanced-stage malignancies. Despite their chance to achieve long-term efficacy, they may induce immune-related adverse events (irAEs). The main irAEs include endocrinopathies, hepatitis, interstitial pneumonia, skin lesions, mucosal inflammation, diarrhea, and colitis. Although most irAEs with severe toxicity are managed with immunosuppressive therapies, occasionally, they can induce infectious disease (e.g., cytomegalovirus infection). This report presents the case of a patient with lung squamous cell carcinoma (SCC) who developed gastrointestinal irAEs, as well as subsequent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and cytomegalovirus infection.

Case report

A man in his seventies was referred to our hospital following the detection of a nodule in the right lung during a routine health examination. Transbronchial biopsy revealed the presence of SCC. (Figure 1A). The examination for epidermal growth factor receptor (EGFR) mutation, anaplastic lymphoma kinase (ALK) translocation, and ROS1 proto-oncogene receptor tyrosine kinase (ROS1) mutations yielded negative results. The tumor proportion score of programmed cell death 1 ligand 1 (PD-L1) was 95% (Figure 1B). The patient was finally diagnosed with stage IIIA lung SCC, and

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received concurrent radiation chemotherapy (i.e., carboplatin + paclitaxel + radiation with 60 Gy in 30 fractions). This treatment was followed by maintenance therapy with durvalumab at 2-week intervals. Six months after completion of first-line chemoradiotherapy, he received additional chemo-

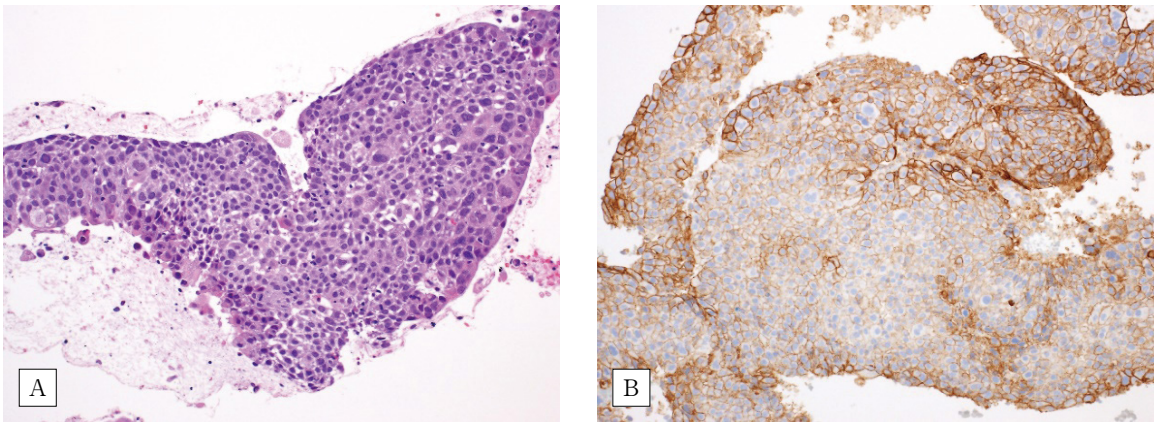

Figure 1 Transbronchial biopsy of squamous cell carcinoma
A) Hematoxylin and eosin staining (× 20).
B) Immunohistochemical staining of PD-L1 (× 20).
PD-L1, programmed cell death 1 ligand 1

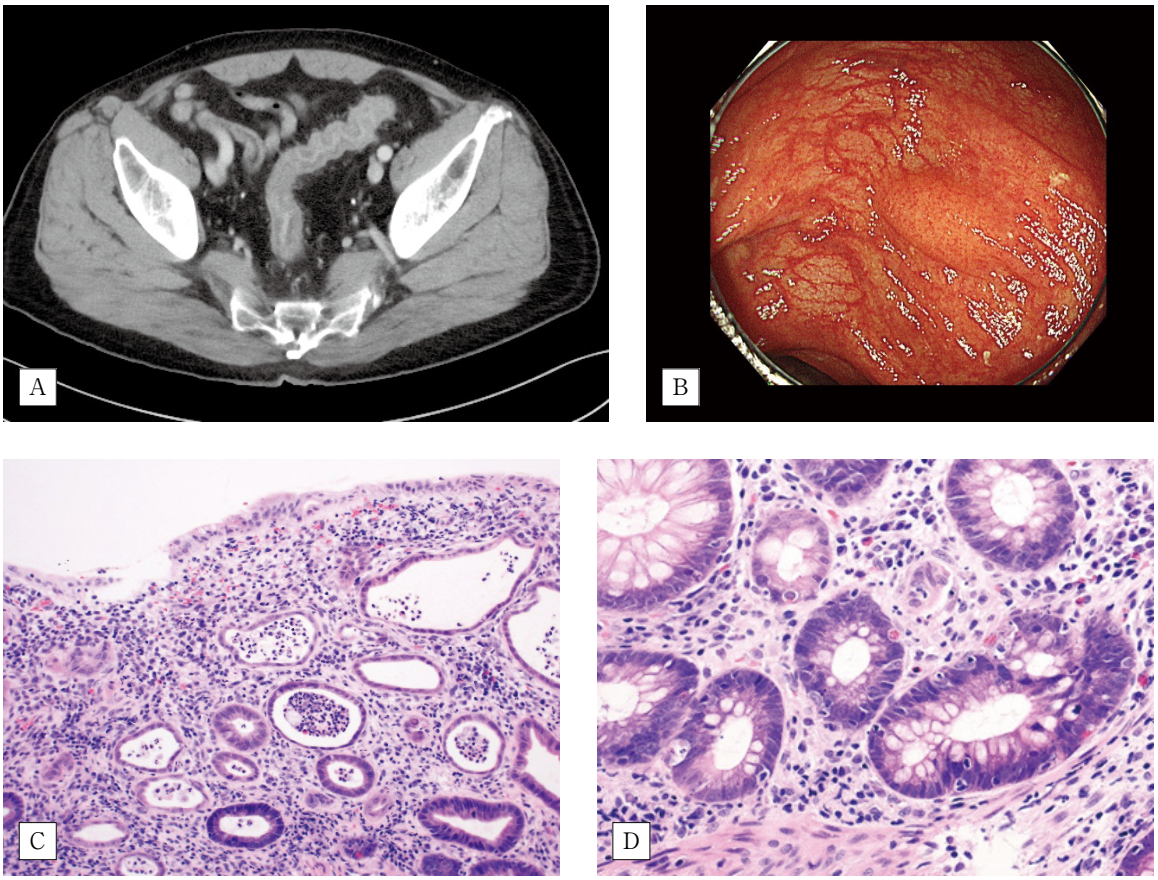

Figure 2 Computed tomography, endoscopy, and histopathological findings of the colon
A) Abdominal computed tomography shows bowel wall thickening.
B) Mucosal erythema.
C) Rectal mucosa with abundant inflammatory cells and atrophic crypt abscess (× 20).
D) Prominent crypt epithelial apoptosis (× 40).

therapy (i.e., nanoparticle albumin-bound paclitaxel + carboplatin + pembrolizumab) for lymph node recurrence. He reported severe diarrhea 10 days after the administration. Computed tomography imaging revealed bowel wall thickening (Figure 2A). He was diagnosed with grade 3 (Common Terminology Criteria for Adverse Events Version 5.0: CTCAE) immune-related colitis and treated with high-dose prednisolone (beginning with 2mg/kg/day and tapering to lower dosage gradually) and infliximab (5mg/kg). Following a limited improvement in abdominal symptoms, he underwent lower endoscopy examination. Colonoscopic findings showed inflammation of the entire colon with a reddish, oedematous mucosa (Figure 2B). Histologically, mixed inflammatory infiltrates with crypt abscesses were observed (Figure 2C). Moreover, increased apoptosis of crypt epithelial cells was observed (Figure 2D). Oral prednisolone (10 mg/day as a maintenance dose) was administered for the treatment of immune checkpoint inhibitor-induced colitis.

The patient was infected with SARS-CoV-2 during radiation therapy for lymph node metastasis at eight-months after colitis onset. For the treatment of SARS-CoV-2 pneumonia, the patient received 20 mg/day prednisolone and remdesivir. Following recovery from SARS-CoV-2, the dose of prednisolone was tapered to 10 mg/day. He complained of abdominal pain after meals, and underwent endoscopy examination that revealed multiple gastric ulcers (Figure 3A). Histologically, a heavy inflammatory cell infiltrate throughout the mucosa was observed (Figure 3B). The presence of intraepithelial CD8-positive lymphocytes (Figure 3C) suggested an irAE. Atypical mesenchymal cells with an intranuclear inclusion body were found (Figure 4A). Immunohistochemical analysis for cytomegalovirus infection was positive (Figure 4B) and blood examination revealed C7-HRP positivity; hence, we the patient was diagnosed with cytomegalovirus infection. The administration of ganciclovir was effec-

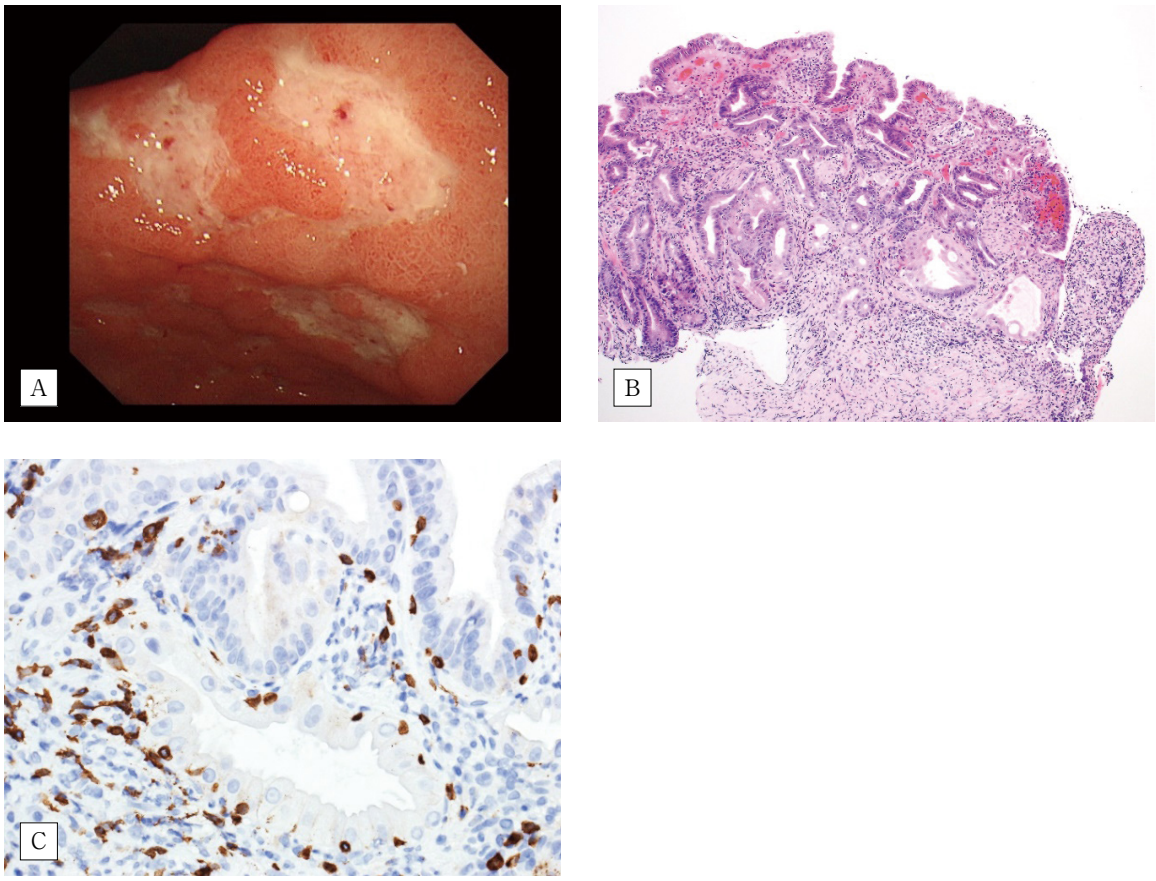

Figure 3 Endoscopy and histopathological findings of the stomach
A) Multiple ulcers.
B) Gastritis with erosion (× 40).
C) CD8 immunohistological staining highlights the lymphocytes (× 40).

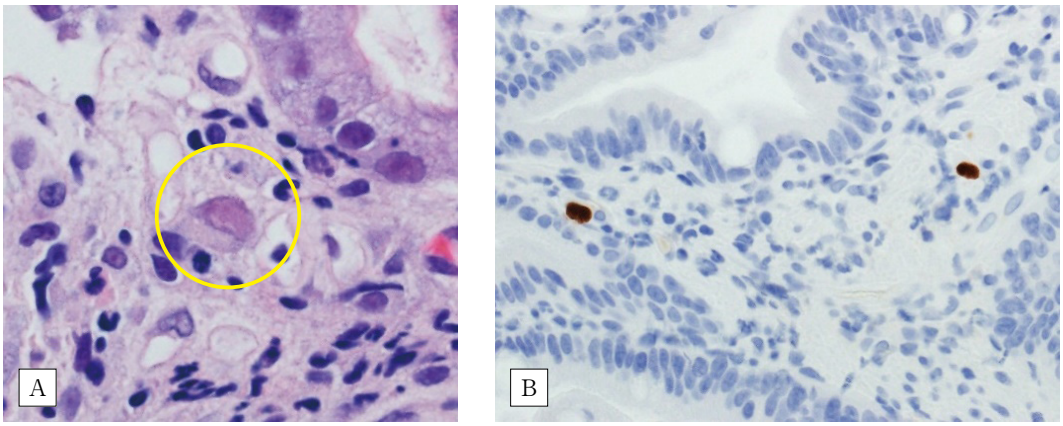

Figure 4 Atypical mesenchymal cells in gastric mucosa
A) Intranuclear inclusion body within the circle (× 60).
B) Immunohistochemical staining with anti-cytomegalovirus antibody (× 40).

tive against cytomegalovirus gastritis.

Written informed consent was obtained from the patient.

Discussion

Immune checkpoint inhibitors are monoclonal antibodies that block inhibitors of T-cell activation and may cause autoimmune manifestations. The incidence of colitis in patients treated with anti-programmed cell death 1/PD-L1 (anti-PD-1/PD-L1) therapy is <5%[1]. Any grade irAEs have been reported to occur 32.9% of lung cancer patients treated with pembrolizumab[2]. In this case, time on pembrolizmab prior to diarrhea was 10days; shorter than median time from PD-1 inhibitor initiation to irAE onset was 3months[3]. The cause might be the first-line durvalumab.The endoscopic findings of intestinal irAE resemble those of ulcerative colitis[4]. Inflammatory changes in the entire colon, as noted in the present case, can be observed in patients with inflammatory bowel disease or infectious disease. The morphological changes associated with intestinal irAE are classified into four categories, namely active colitis with apoptosis, lymphocytic colitis, acute self-limiting colitis, and collagenous colitis[5,6]. The histopathologic differential diagnoses of intestinal irAE include inflammatory bowel disease, infectious disease, and other therapeutic effects[4,5]. Intraepithelial CD8-positive lymphocytosis is a key component in the pathogenesis of irAEs[7].

The development of an irAE in immunosuppressed patients is associated with cytomegalovirus infection. Although there are some theories[8-10], the risk of SARS-CoV-2 infection in patients receiving immune checkpoint inhibitors is currently unclear. A study demonstrated that the use of corticosteroids and/or anti-TNF drugs was a major risk factor for the development of infection among patients with melanoma who received immune checkpoint inhibitors[11]. In this case, it appears that the immunosuppressive agents contributed to SARS-CoV-2 and cytomegalovirus infection. Rectal biopsy using immunohistochemistry, performed at the time of discontinuation of prednisolone or infliximab, was negative for cytomegalovirus. Cytomegalovirus infection should be considered in cases in which a patient with an irAE develops resistance to immunosuppressive therapy. Eroded or cytomegalovirus-infected mucosa sometimes revealed atypical mesenchymal cells which should be distinct from malignancy. The distinction between gastrointestinal irAE and infection is important, because the treatment modalities for these conditions differ considerably. Sufficient clinical information is warranted for accurate pathological diagnosis.

As shown in this report, gastric irAE and cytomegalovirus infection can occur simultaneously during the treatment of colonic irAE. Hence, we should take notice of complication of irAEs and virus infection.

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Author contributions

AH, HI, ST, and TY performed the histological evaluation; TO, SS performed data analysis and interpretation; AH, ST wrote the manuscript; and all authors approved the final manuscript.

Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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Case reports

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Case Report and Minireview of the Literature on Blunt Azygos Injury

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Azygos vein injury seems to be an uncommon cause of hemothorax and hemomediastinum; however, this injury is potentially fatal. We report a fatal case of blunt azygos injury and a PubMed search was undertaken to identify English articles from 1989 to 2022 using the key words "azygos", "injury" and "blunt". We found 28 articles about blunt azygos injury and 39 patients including the present case (average 41.2 years [range: 18–81 years]; male, n=20; female, n=19). The other variables were as follows: right hemothorax (n=32); unstable circulation on arrival (n=32); and survival (n=19; unknown, n=2). These cases were divided into two groups based on the outcome: the survival group and the fatal group. There were no significant differences with regard to the year of the report, age, sex, rate of right rib fracture, rate of preoperative computed tomography (CT) examination, rate of associated injury, and rate of operation. The rate of shock on arrival in the survival group was significantly lower than that in the fatal group. The rate of azygos arch injury in the survival group was significantly greater than that in the fatal group. The emergency physician must consider azygos vein injury as a possible cause of right hemothorax when a patient with blunt chest trauma presents persistent hypotension.

Key words: trauma, shock, azygos injury, outcome

Introduction

The azygos vein is located on the right side of the vertebral column and penetrates from the retroperitoneum through the diaphragm to join the superior vena cava at the T4 level[1)]. Fracture-dislocation of the mid-thoracic spine or ribs, as a result of blunt thoracic trauma, can tear the azygos vein[1)]. The vein can also be torn, in the absence of skeletal injuries, by horizontal acceleration/deceleration forces[1)]. Most reports of blunt trauma to the azygos vein in the relevant literature are related to motor vehicle collisions[1)]. Patients frequently present with shock-like symptoms and expanding hemothorax, necessitating prompt surgical repair[1–28)]. Azygos vein injury seems to be an uncommon cause of hemothorax and hemomediastinum; however, this injury is potentially fatal. We herein report a fatal case of blunt azygos injury and a review of the relevant literature. The protocol of this retrospective study was approved by Juntendo Shizuoka Hospital review board (approval number: 298). We obtained oral informed consent from the bereaved.

Case presentation

A 63-year-old man fell from a 2nd floor veranda while leaning over a banister trying to catch a ladder. When emergency medical technicians checked him, he was in shock state with consciousness disturbance; thus, he was transported to our emergency room (ER) by ambulance within 20 minutes. He had a medical history of diabetes mellitus and colon cancer. On arrival, his vital signs

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were as follows: Glasgow Coma Scale, E4V3M6; blood pressure, 75/− mmHg; heart rate, 140 beats per minute; respiratory rate, 30 breaths per minute and percutaneous saturation, 98% under 10 L per minute of oxygen. A physical examination revealed a head contusion and weakness of the right respiratory sound. The chest roentgenography showed decreased radiolucency in the right lung field (Figure 1), suggesting right hemothorax. Focus assessment of sonography for trauma also showed fluid collection, which was limited to the right thoracic cavity. Initially, he underwent immediate massive transfusion without cross-matching and tracheal intubation following right thoracostomy, which drained over 1 L of hemorrhaging. As his blood pressure did not respond to massive transfusion, right thoracotomy was tentatively performed by young emergency physicians in order to pack gauze and achieve hemostasis around the pulmonary hilus, where blood was emerging without a hilar clamp, while the patient was in the supine position. However, his unstable circulation deteriorated. After closing the thoracotomy, he was moved to the computed tomography (CT) room and CT revealed hemorrhaging from the inferior azygos vein near a thoracic vertebral fracture (Figure 2) and right subdural hematoma. He experienced cardiac arrest after returning to the ER. A thoracic surgeon standing by at home attended the ER and explored the right thoracic cavity by opening the thoracotomy. The surgeon recognized an azygos arch injury and achieved hemostasis by gauze packing. The surgeon also performed manual

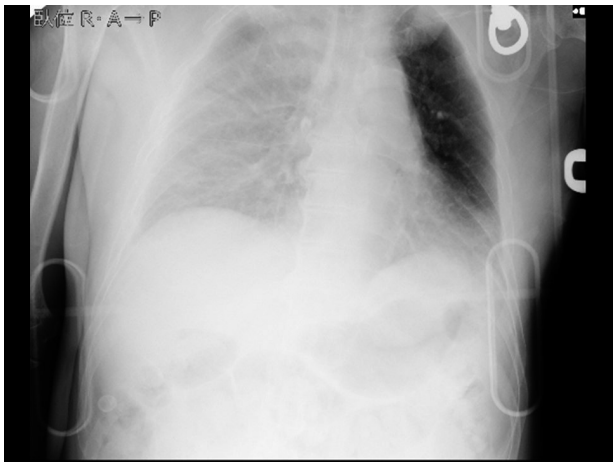

Figure 1 Chest X-ray on arrival
The X-ray suggested right hemothorax.

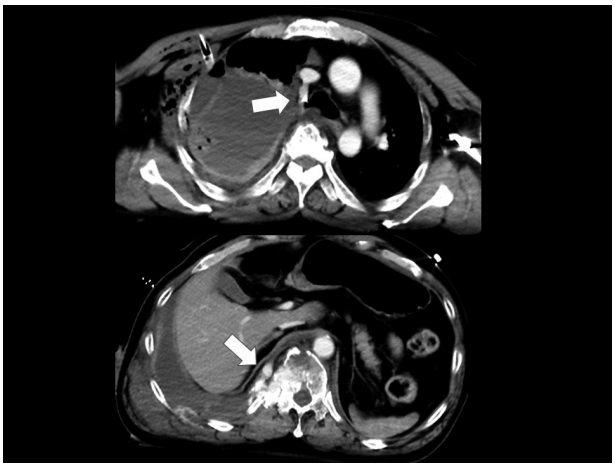

Figure 2 Enhanced chest computed tomography after tentative thoracotomy
Bleeding from the azygos arch was controlled (upper arrow) but hemorrhaging from the inferior azygos vein near the thoracic vertebral fracture remained (lower arrow).

compression at the hemorrhaging site of the inferior azygos vein, and transfusion was continued. However, a return of spontaneous circulation was not obtained due to hemorrhaging associated with the trauma itself and the operative incision site due to the patient's bleeding tendency.

Review and analysis of the relevant literature

A PubMed search was undertaken to identify English articles from 1989 to 2022 using the key words "azygos", "injury" and "blunt". We found 28 articles about blunt azygos injury[1–28]. We summarized these cases, including the present case, in Table 1. We also added the report by Wall et al. into Table 1 as a supplement, which described the treatment of the largest series of penetrating azygos injury cases in the relevant literature[29]. There were 39 cases of the blunt azygos injury (average age, 41.2 years [range: 18–81 years]; male, n=20; female, n=19). The mechanisms of injury were as follows: traffic accident (n=29); fall (n=4), falling object (n=1), assault (n=1), sports (n=1), chest compression for cardiac arrest (n=1), and unknown (n=2). The other variables were as follows: right hemothorax (n=32; unknown, n=1); unstable circulation on arrival (n=32; unknown, n=1); right rib fracture, (n=20; unknown, n=5); preoperative CT examination (n=12), associated injury (n=30; unknown, n=1); surgical operation (n=36; unknown, n=2); and survival (n=19; unknown, n=2). While, a delayed appearance of

Table 1 Summary of case of blunt azygos injury

No	Name	Refe-rence	year	age	sex	mechanism	right hemo-thorax	shock	rib fracture	site	CT	associated injury	Ope-ration	Sur-vival
1	Li	1	2022	81	female	traffic accident	yes	yes	yes	arch	no	diaphragma, liver, omentum, pelvis	yes	yes
2	Li	1	2022	38	male	traffic accident	yes	yes	yes	arch	no	Th3, L4, pelvis	yes	yes
3	DeMaio	2	2021	28	female	traffic accident	yes	no	yes	arch	yes	Th4,5, sternum	yes	yes
4	Laohathai	3	2019	33	female	traffic accident	yes	no	no	arch	no	radius	yes	yes
5	Papadomanolakis	4	2016	28	female	traffic accident	yes	yes	no	?	no	liver, femur	yes	no
6	Papadomanolakis	4	2016	50	male	traffic accident	yes	yes	yes	?	no	Th, liver, pelvis	yes	no
7	Papadomanolakis	4	2016	28	male	traffic accident	yes	yes	yes	?	no	femur	yes	no
8	Papadomanolakis	4	2016	35	male	traffic accident	yes	yes	yes	?	no	liver	yes	no
9	Papadomanolakis	4	2016	41	male	fall	yes	yes	yes	?	no	liver, spleen	yes	no
10	Papadomanolakis	4	2016	20	male	traffic accident	yes	yes	yes	?	no	liver, kidney, femur	yes	no
11	Papadomanolakis	4	2016	65	female	traffic accident	yes	yes	yes	?	no	pelvis	yes	no
12	Yang	5	2014	52	female	chest compression	yes	yes	yes	?	yes	none	yes	no
13	Mohajeri	6	2014	45	male	sport	no	no	no	arch	yes	none	no	yes
14	Haq	7	2014	52	male	traffic accident	?	?	?	arch	yes	?	?	?
15	Cao	8	2012	60	male	falling object	yes	yes	yes	arch	yes	lumbar, lower extremity	yes	yes
16	Juraszyński	9	2010	70	female	blunt	no	no	no	SVC junction	yes	none	yes	yes
17	Endara	10	2010	21	male	traffic accident	yes	yes	?	SVC junction	no	spleen	yes	no
18	Drac	11	2007	22	male	traffic accident	yes	yes	yes	?	yes	head, spleen	yes	no
19	Kamiyoshihara	12	2007	71	female	traffic accident	yes	?	yes	arch	yes	kidney, leg	yes	yes
20	Nguyen	13	2006	21	male	traffic accident	yes	yes	?	Th4	no	femur, descending aorta	yes	yes
21	Bowles	14	2000	21	male	traffic accident	yes	yes	?	arch	yes	none	yes	yes
22	Sharma	15	1999	75	female	traffic accident	yes	yes	yes	arch	no	liver, head, pelvis	yes	yes
23	Sugimoto	16	1998	44	male	traffic accident	no	yes	no	arch	no	abdomen	yes	no
24	Cagini	17	1998	18	female	traffic accident	yes	yes	no	arch	no	omentum	yes	yes
25	No author	18	1996	51	female	blunt	yes	no	no	hemidiaphragm	yes	none	?	?
26	Butler	19	1995	23	male	traffic accident	yes	yes	yes	arch	no	head, Th3,4, spleen, tibia	yes	yes
27	Jain	20	1994	51	female	assault	yes	yes	no	Above diaphragm	no	none	yes	no
28	Inoue	21	1993	41	female	traffic accident	yes	yes	no	SVC junction	no	none	yes	yes
29	Walsh	22	1992	41	male	fall	yes	yes	no	SVC junction	no	none	yes	no
30	Thurman	23	1992	19	male	traffic accident	yes	yes	?	arch	no	head, ankle	yes	yes
31	Shkrum	24	1991	23	male	fall	no	yes	no	Th5	yes	Th, head	yes	no
32	Shkrum	24	1991	39	female	traffic accident	no	yes	no	Th4	no	Th, head, liver, spinal cord	yes	no
33	Shkrum	24	1991	48	female	traffic accident	yes	yes	yes	Th4	no	Th, liver, spleen	yes	no
34	Shkrum	24	1991	24	female	traffic accident	no	yes	no	Th4–5	no	pelvis	yes	no
35	Baldwin	25	1984	28	female	traffic accident	yes	yes	yes	SVC junction	no	abdomen, lower extremities	yes	yes
36	Sherani	26	1986	25	male	traffic accident	yes	yes	no	Th4–5	no	head, abdomen, lower extremities	yes	yes
37	Coates	27	1987	63	female	traffic accident	yes	yes	yes	SVC junction	no	head, abdomen, lower extremities	yes	yes
38	Snyder	28	1989	52	female	traffic accident	yes	yes	yes	Inferior SVC junction	no	spine	yes	yes
39	Present case		2021	63	male	fall	yes	yes	yes	arch, Th12	yes	head	yes	no
	Wall	29	2006	?	?	gun shot 19, stab 3	?	?	?	?	?	Multiple	yes	mortality 36%

CT: computed tomography, Th: thracic spine, L: lumbar spine, SVC: superior vena cava

right hemothorax after blunt chest trauma due to traumatic azygous vein injury, likely from rupture of a pseudoaneurysm, was observed. Thus, the diagnosis of azygous vein injury without initial hemothorax can be hampered in extremely rare case[16]. These cases were divided into two groups: the survival group (n=19), which included cases in which the outcome was survival; and the fatal group (n=18), which included cases who died. The characteristics of the cases were compared between the two groups, including the year of the report, age, sex, rate of shock on arrival, rate of right rib fracture, rate of azygos arch injury, rate of preoperative CT, rate of associated injury, and rate of operation. The chi-squared test, median test or non-paired Student's *t*-test were used for the statistical analyses. P values of <0.05 were considered to indicate statistical significance. The results of the analysis are shown in Table 2. There were no statistically significant differences with regard to the year of the report, age, sex, rate of right rib fracture, rate of preoperative CT examination, rate of associated injury and rate of operation. The rate of shock on arrival in the survival group was significantly lower than that in the fatal group, and the rate of azygos arch injury in the survival group was significantly greater than that in the fatal group.

Discussion

This review of cases of blunt azygos injury is the first report to suggest that shock on arrival and the location of azygos vein injury may have an influence on final outcome of the patient.

Shock on arrival in patients with blunt trauma suggests massive bleeding from injured sites and/or spinal cord injury, and previous reports have also demonstrated that shock on arrival is a poor prognostic factor[30, 31]. Accordingly, ER physicians must consider azygos vein injury as a possible cause of right hemothorax in patients with blunt chest trauma who show persistent hypotension. The reason for the favorable outcome of azygos arch injury in comparison to other sites might be that it is easier to visually recognize the injured site. Usually, trauma patients are managed in supine position in the ER and tentative thoracotomy is also performed in the same position because subsequent tentative laparotomy might be required to explore abdominal injuries[32]. The azygos arch was easily visually recognized in the supine position, however, other sites might be hidden by the pulmonary hilus, lung or diaphragm[33]. In the present hemostasis at the site of the azygos arch injury was obtained by direct gauze packing; however, the packing at the inferior injury site of the azygos vein was insufficient.

This review of cases of blunt azygos injury failed to show that recent medical development has resulted in favorable outcomes. Recent surgeons are familiar with using preoperative radiological studies to perform a planned operation precisely, safely and less invasively. In contrast, experienced trauma surgeons can perform urgent surgical operations without radiological studies, with manual intraoperative exploration to identify the site of bleeding and apply hemostasis[34]. Advanced Trauma Life Support® (ATLS®) does not recommend that trauma patients with unstable circulation be moved

Table 2 Comparison between the survival and fatal groups

	Survival n = 19	Fatal n = 18	p value
Year of the report	2000	2012	0.24
Age	42.7 ± 21.3	38.6 + 14.3	0.77
Sex (male/female)	8/11	11/7	0.24
Shock (%)	14 (73)	18 (100)	0.01
Right rib fracture (%)	10/n=16 (62)	10/n=17 (58)	0.82
Arch (%)	12 (63)	1/n=9 (11)	0.006
CT (%)	6 (46)	4 (22)	0.52
Associate injury (%)	15 (78)	15 (83)	0.73
Operation (%)	18 (94)	18 (100)	0.24

CT; computed tomography

to a CT room or for CT examination to be used to identify sites of bleeding[35]. However, it is important for hemostasis to be immediately achieved at hemorrhaging sites in patients with unstable circulation. The number of patients with severe trauma has been decreasing year by year, and the number of experienced trauma surgeons in Japan has declined[36]. The fact that the diagnostic studies included as part of the initial ATLS® trauma survey are not well equipped to diagnose such a fatal vascular injury[37]. In addition, recent studies showed the efficacy of evaluation using whole CT during resuscitation in the hybrid ER, for even trauma patients with unstable circulation, in order to detect sites of hemorrhaging and facilitate the immediate performance procedures to obtain hemostasis[38–41]. Accordingly, to increase the survival rate of patients with fatal vascular injuries, such as blunt azygos injury, the early recognition of the site of hemorrhaging using CT and the immediate execution of surgical hemostasis in an appropriate position for modern surgeons (less experienced in the management of severe trauma) may be required, even when initial fluid resuscitation fails and unstable circulation remains.

Regarding what measures should be taken by young physicians in a standard hospital without a hybrid ER to obtain a survival outcome in patients with fatal azygos injury and unstable circulation until veteran surgeons arrive. Aside from the ATLS® protocol, a 1:1:1 ratio of packed red blood cells, fresh plasma, and platelets with minimal crystalloids is the preferred resuscitative strategy to avoid diluted coagulopathy by crystalloid fluid resuscitation[42]. Recently, in patients experiencing hemorrhagic shock, whole-blood transfusion was reported to be associated with both an improved survival and decreased overall blood utilization[43]. If a patient does not obtain stable circulation even after massive transfusion, they should be intubated to secure the airway[35]. After definitively securing the airway, a CT examination should be considered, although the proper timing of CT remains controversial[44]. A chest drain is usually inserted to drain the hemothorax and evaluate the volume in order to decide the timing of radical operation. Tentative drain clamping may be effective for achieving hemostasis at the bleeding source or reducing the total hemorrhaging volume by the hematoma tamponade effect, based on our personal experience and evidence from total knee arthroplasty[45]. However, it should be noted that drain clamping may result in hemorrhagic death or fatal tension hemothorax. Intensive hypotensive resuscitation is recommended, as it is safe and has a lower mortality rate than normotensive resuscitation in hemorrhagic shock patients. There is also less blood loss, hemodilution, ischemia, and hypoxia in tissues with such an approach[46]. If young physicians aggressively attempt damage control intervention using right thoracotomy but fail to identify the bleeding source, hilar clamping or twisting may be attempted to detect the bleeding source[47, 48]. If the bleeding cannot be stopped with these procedures, the bleeding source likely lies outside of the pulmonary artery and venous system. In addition, in cases with an unknown bleeding source, a large amount of gauze should be packed blindly in order to achieve hemostasis[49]. Alternatively, clam-shell thoracotomy may be useful for identifying the bleeding source, even in the supine position[50].

Conclusion

We presented a fatal case of blunt azygos injury and the results of an analysis of the relevant literature. ER physicians must consider azygos vein injury as a possible cause of right hemothorax in patients with blunt chest trauma if the individual shows persistent hypotension. In addition, the early recognition of the site of hemorrhaging using CT may be required, even if the patient's circulation remains unstable after initial fluid resuscitation.

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Author contributions

KM was a major contributor in writing the manuscript. KJ, SH and YY were editing the manuscript. All authors read and approved the final manuscript.

Conflicts of interest statement

We do not have conflict of interest to declare.

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Case Reports

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Lessons Learned in Practice with Li-Fraumeni Syndrome: LFS-Related Breast Cancer Treatment Strategy and Establishment of a Surveillance System

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We herein present the case of a 33-year-old woman with no family history of metachronous bilateral breast cancer and osteosarcoma, diagnosed with Li-Fraumeni syndrome (LFS), which is a rare autosomal dominant hereditary cancer syndrome associated with a germline *TP53* variant. She was diagnosed with left distal femoral osteosarcoma at the age of 16, and metachronous bilateral breast cancer at the ages of 29 and 33. When the third cancer was diagnosed, a hereditary tumor syndrome was suspected and the patient was referred to our genetic outpatient clinic. There was no family history of the 'core' cancers for LFS, but since the patient met Chompret's criteria, germline *TP53* genetic testing was performed with the patient's will. A pathogenic variant, *TP53*:c.216dupC (p.Val73ArgfsX76) was found in exon 4 of the gene. This case is didactic because radiotherapy was performed on the first breast cancer before the diagnosis of LFS was made; radiation should be avoided if there are other options in LFS because of the inability to repair DNA damage. As a lesson learned, oncologists reaffirmed the importance of being aware of hereditary tumors from the keywords "multiple," "young," "familial," and "rare," and consulting the genetic department. In addition, surveillance using whole-body magnetic resonance imaging is recommended in LFS. However, this system is not yet provided nationwide, but we have newly settled it in our hospital.

Key words: Li-Fraumeni syndrome, hereditary cancer syndrome, treatment management, surveillance, whole-body MRI

Introduction

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant hereditary cancer syndrome associated with germline *TP53* pathogenic or likely pathogenic variants[1)]. The tumor suppressor gene, *TP53*, is located on chromosome 17, and the protein product of *TP53* is localized in a cell nucleus and binds directly to DNA. It has been called the "guardian of the genome" and plays important roles in controlling the cell cycle and apoptosis[2)]. The frequency of germline *TP53* variants in the general population has been reported to be about 1.6% in pediatric cancer patients[3, 4)] and about 0.2% in adult cancer patients[5)]. The penetrance of germline *TP53* variants is 75% in males and almost 100% in females[6)]. Regarding age of cancer onset, an analysis of 415 individuals with *TP53* pathogenic variant in 214 French families with LFS showed that the cancer penetrance for young people aged 0, 5, and 18 years were 4%, 22%, and 41%, respectively[6)]. Furthermore, an analysis by the US National Cancer

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Institute of 286 *TP53* pathogenic variants in 107 families with LFS showed that the 50% cumulative cancer onset age was 46 years for male and 31 years for female[7]. It has been reported that 12.2 % of cases are de novo LFS; therefore, cases without a family history are scattered[8]. LFS has a wide tumor spectrum, including the so-called 'core' LFS cancers: soft-tissue sarcomas, osteosarcomas, adrenocortical carcinomas, central nervous system tumors, and very-early onset female breast cancer[9]. Juvenile onset of cancer and multiple onsets and types of cancers in one patient are characteristics of LFS. Clinical guidelines for the management of LFS have been published in various countries, including Japan[10].

According to a report on the analysis of hereditary breast cancer-related genes in Japanese breast cancer patients, the percentage of patients with germline *TP53* variants is 3.9%, which is small compared to 72.5% for germline *BRCA1/2* variants[11]. Therefore, LFS is rarely encountered in breast cancer treatment, and it is not easy to make a diagnosis. However, in the treatment of breast cancer, radiation therapy (RTx) is often used concomitantly, and the relative contraindication in this disease should be taken into consideration when making treatment decisions[12]. This report describes a case of LFS that was suspected by its history and clinical course, despite no LFS-related family history of hereditary tumors, which led to LFS genetic testing.

Case report

A 33-year-old woman was diagnosed with distal left femoral osteosarcoma at the age of 16 due to pain in the left knee. She received perioperative chemotherapy and local excision and has had no signs of recurrence until now. At the age of 29, a left breast mass was detected by ultrasound screening and diagnosed as breast cancer. She underwent breast-conserving surgery and sentinel node biopsy, and was diagnosed with pT1bN0M0, Stage I, invasive micropapillary carcinoma (5×4 mm; estrogen receptor [ER] 60%, progesterone receptor [PgR] 90%, human epidermal growth factor receptor 2 [HER2] 3+, Ki67 70%; surgical margin positive at the lateral margin for in situ component). She received weekly paclitaxel+trastuzumab, LH-RH agonist, tamoxifen and RTx as postoperative therapy. Four years after the surgery for left breast cancer, at the age of 33, calcification of her right breast was detected on mammography. A stereotactic core biopsy was performed and diagnosed as breast cancer, cTisN0M0 Stage 0 (ductal carcinoma in situ [DCIS]). When she was diagnosed with metachronous contralateral breast cancer, a hereditary tumor was suspected and she was referred from her family clinic to our genetic department. Her family history included a maternal grandfather with colorectal cancer at the age of 78 and a paternal uncle with lower leg suspected skin cancer at 50 years (Figure 1). This patient had no obvious LFS-related family history. However, since the patient met Chompret's criteria (2015, Table 1) with multiple cancers and early-onset breast cancer in the 'core' tumors, we performed germline *TP53* genetic testing (FALCO Ltd., Kyoto, Japan), with her consent (written informed consent was obtained from the patient.). The genetic test results revealed a pathogenic variant, TP53:c.216dupC (p.Val73ArgfsX76) was found in exon 4 of the gene, and a diagnosis of LFS.

The patient was referred to the department of breast surgery through the genetic department for further treatment. At the same time as the right mastectomy, the patient preferred to have a left residual mastectomy because of the positive DCIS margins at the previous surgery and for risk reduction. She underwent bilateral mastectomy and breast reconstruction. The postoperative pathology results were as follows. The right breast cancer was DCIS solid > cribriform type, 3×2×12 mm, NG2, pTisN0M0, Stage 0 (ER-, PgR-, HER2 3+, Ki67 40%). The left breast was assessed in every 1 cm-slice for the entire section, due to the residual mastectomy being for risk reduction. Close to the previous surgical scar, DCIS was found (tumor size: 12×6×20 mm, comedo type, NG2, pTisNxMx), which was considered to be a residual lesion due to its similar histology. Additionally, p53 immunohistochemical staining (monoclonal, anti-mouse, clone PAb1801) was negative (Figure 2). Sporadic breast cancer cases in the control group that were in situ breast cancer and of similar subtype also tested negative.

Currently, she is receiving endocrine therapy for left breast cancer and will be followed up in our department for 5 years after surgery, including

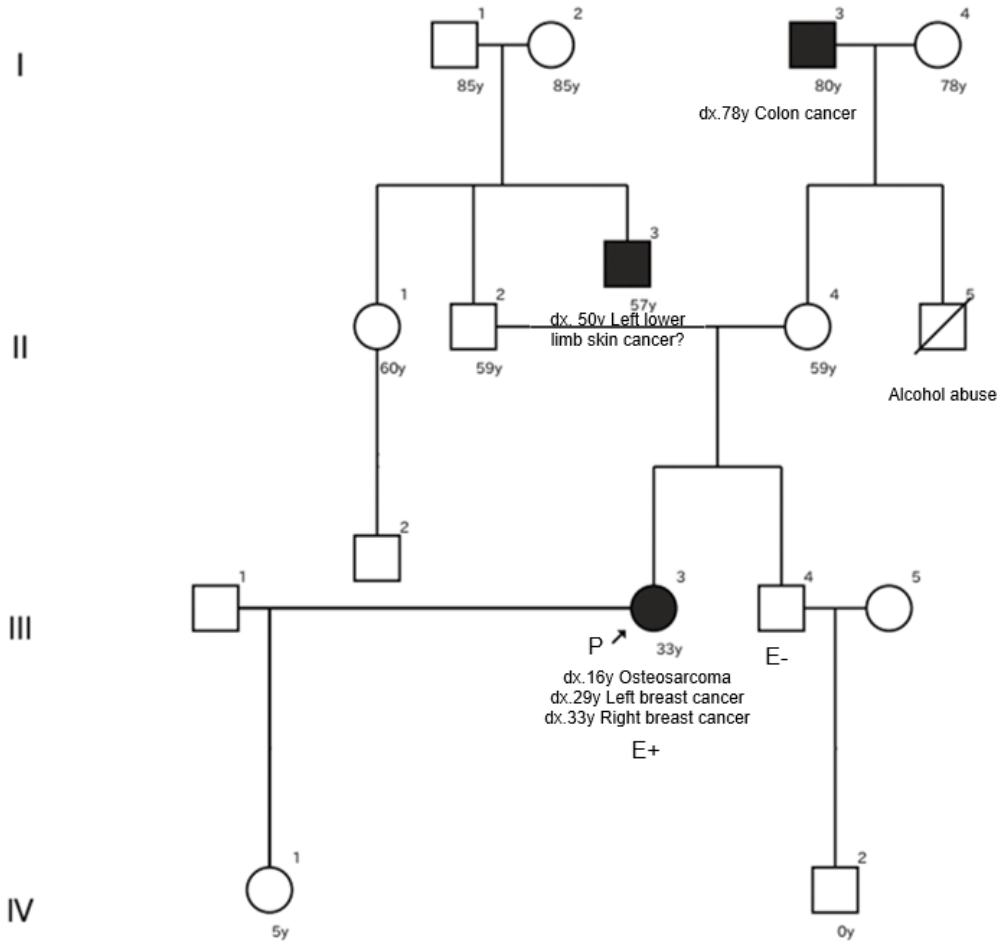

Figure 1 Family history
Dx, age at diagnosis; d, age at death; y, years old.

Table 1 2015 version of Chompret's criteria for Li-Fraumeni syndrome diagnosis

Familial presentation	Proband with TP53 core cancer before 46 years and at least one first- or second-degree relative with a core tumor before 56 years.
Multiple primitive tumors	Proband with multiple tumors, including two TP53 core tumors, the first of which occurred before 46 years, irrespective of family history.
Rare tumors	Patient with adrenocortical carcinoma, choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history.
Very early-onset breast cancer	Breast cancer before 31 years, irrespective of family history.

blood tests and chest wall ultrasound. In contrast, LFS requires lifetime cancer surveillance. Since the surveillance for osteosarcoma has been performed by semi-annual chest and local X-rays, we decided to introduce a surveillance program according to the Toronto protocol[13], which avoids radiation exposure and follow-up drop outs. Through this case, we have just settled a system of annual whole-body magnetic resonance imaging (MRI) examinations in cooperation with the radiology department (Figure 3). In addition, we decided to conduct colonoscopy and abdominal ultrasound every one to two years in cooperation with her family clinic (Figure 4).

This case is speculated be *de novo* onset of LFS because there is no LFS-related family history and due to the high penetrance of the syndrome; the parents have no history of abnormal findings on annual examinations to date. The younger brother underwent carrier diagnosis and no variants were detected. Hereafter, the patient is also considering carrier diagnosis for her 5-year-old daughter, taking

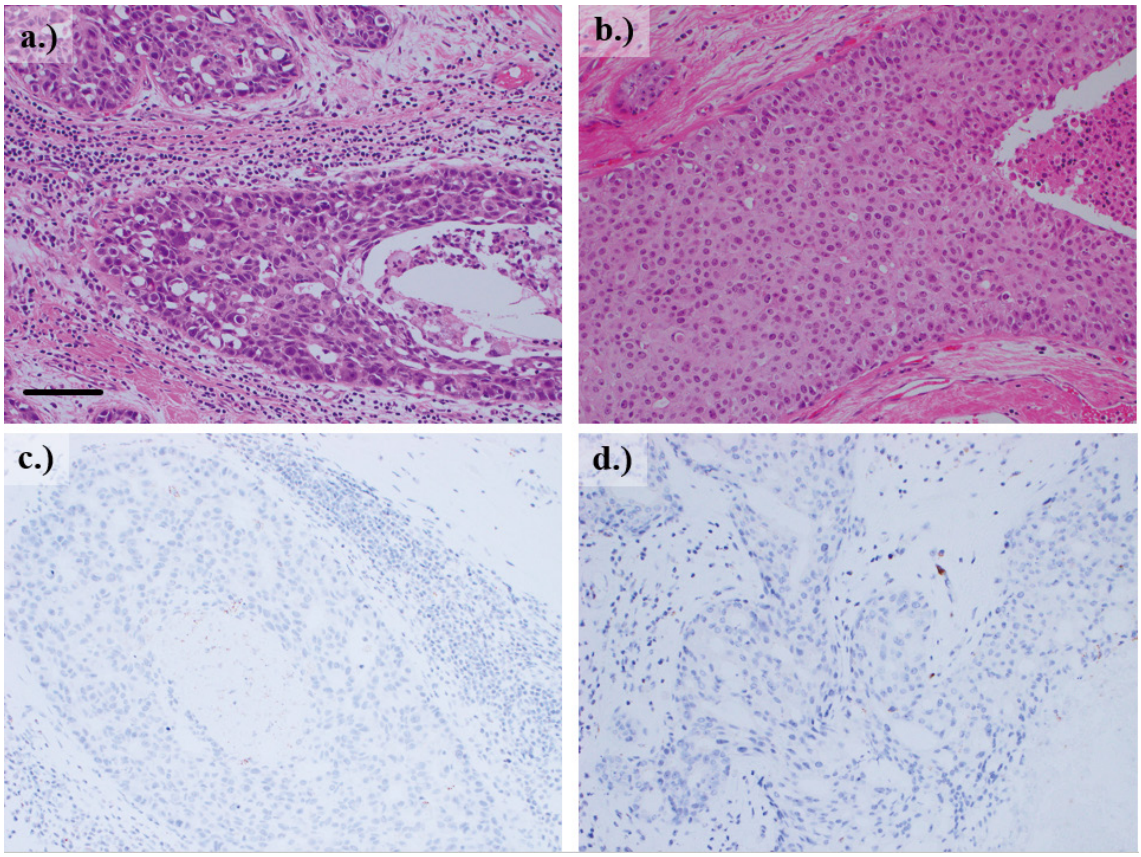

Figure 2 Pathological features of the surgical specimens
a) and c) right breast cancer; b) and d) left breast cancer. a-b) hematoxylin and eosin staining ×100; b-d) p53 staining x100.

into account the advantages and disadvantages.

Discussion

Characteristics of LFS-related breast cancer

Breast cancer is the most common LFS-related tumor, accounting for 27% to 31% of all reported LFS tumors[6]. LFS-related breast cancer is characterized by a median age as young as 33 years, and almost all cases occur before menopause. Among younger breast cancer patients under 30 years, germline *TP53* variants were detected in about 4%-8% of cases (without germline *BRCA1/2* pathogenic variants). There are few reports on clinicopathological characteristics; one report compared 30 LFS breast cancers with 79 sporadic cases and showed a significantly higher rate of HER2 positivity in the LFS group (67% vs. 25%, respectively; $p<0.001$)[14]. In addition, 65% of LFS breast cancers were bilateral. Our case had similar pathological characteristics and clinical course.

Radiation exposure in LFS

LFS-related breast cancer treatment strategies, such as radiation, surgery and drug therapy, are listed in Table 2 according to consensus recommendations[12]. The *TP53* gene is the most important tumor suppressor gene in preventing cancer development. It plays an important role in cell cycle regulation and apoptosis by providing cells with the ability to respond to and repair DNA damage after cellular stress and by triggering multiple downstream repair pathways. Therefore, radiation exposure, which increases the risk of developing a second cancer, should be avoided if there are other options in LFS because DNA damage cannot be repaired. In the present case, LFS was not suspected at the time of the initial breast cancer, and treatment and surveillance with RTx and X-rays were performed. It is not easy for a non-geneticist to suspect a hereditary tumor, other than hereditary breast-ovarian cancer syndrome (HBOC). However, it may be necessary to suspect the possibility of a background hereditary tumor related to breast

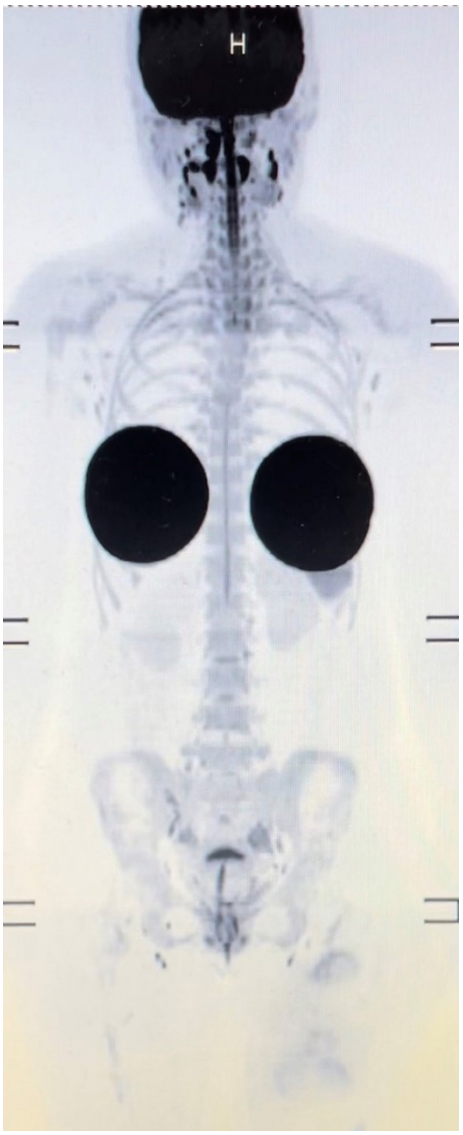

Figure 3 Representative image of whole-body magnetic resonance imaging surveillance

cancer, as it may affect treatment choices.

Meanwhile, there are cases where the benefits to the patient exceed the harms in cancer treatment. The decision of whether or not to prescribe RTx in LFS patients with cancer relies on a delicate multidisciplinary assessment of the risk of a second cancer (based on age and pathogenic variants, or whether heterozygous or in a state) and the oncological prognosis[15].

Surgical treatment in LFS

Total mastectomy should be the surgical treatment of choice for LFS. As for risk-reducing strategies, in HBOC, contralateral risk-reducing mastectomy (CRRM) is covered by insurance. However, CRRM is not recommended for breast cancer patients who have a mutation in a moderate-penetrance breast cancer gene, and therefore an application to an ethics committee is required. As a rare disease, it is difficult to establish evidence regarding LFS, but we believe that it is necessary to provide medical care based on individual requests. In this case, the patient had a positive DCIS margin, which made it appropriate to perform a residual mastectomy[16]. The local recurrence rate is twice as high when the margin of DCIS is positive as when it is negative, and half of the patients with DCIS recurrence will have invasive cancer, so complete resection is recommended.

Our case underwent reconstruction, and in recent years there have been increased warnings about breast-implant-associated anaplastic large

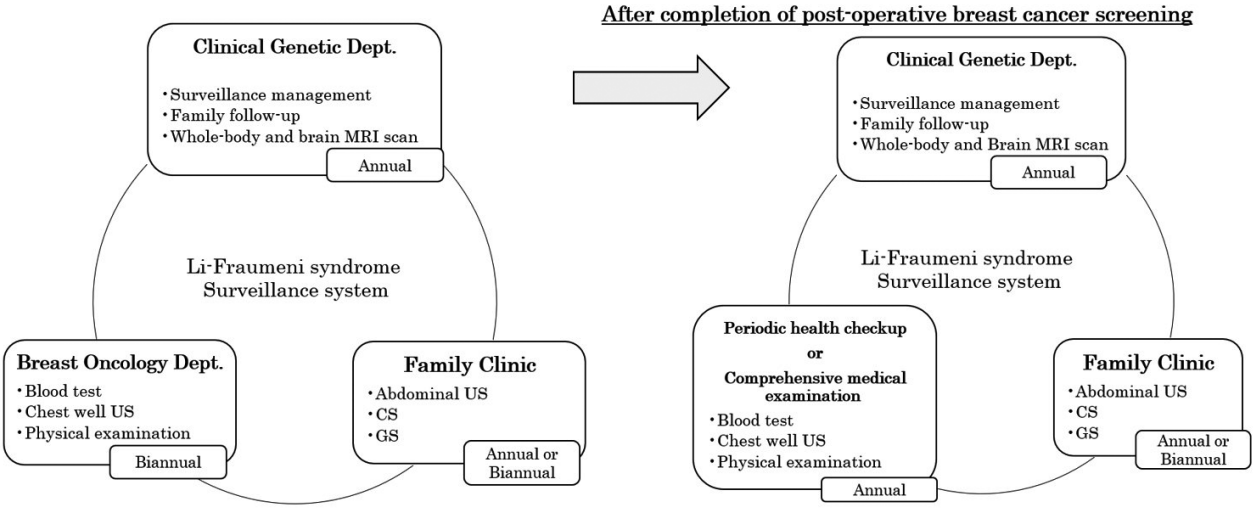

Figure 4 Conceptualization of a long-term Li-Fraumeni syndrome surveillance system to be conducted in collaboration among departments. US, ultrasound; CS, colonoscopy; GS, gastroscopy

Table 2 Consensus recommendations for the management of Li-Fraumeni syndrome[12)]

Radiation therapy	RT of the intact breast is contraindicated. Postmastectomy RT should only be considered in patients with a significant risk of locoregional regional recurrence. 〔Strength of recommendation: Moderate, Quality of evidence: Low (case-series only)〕
Surgical therapy	A mastectomy is the recommended therapeutic option.
Systemic drug therapy	Avoid cytotoxic anticancer drugs that induce DNA damage if possible. PARP inhibitors: insufficient evidence for moderately penetrant genes including TP53.
Diagnostic Imaging	Avoid radiation exposure (e.g.: ultrasound, MRI).

MRI, magnetic resonance imaging; RT, radiation therapy.

cell lymphoma (BIA-ALCL), which is a rare form of T-cell lymphoma that occurs in some people who have had breast implants. BIA-ALCL in LFS has been accumulating and also requires attention[17)]. In this case, reconstruction was performed using a smooth type of implant, but there is a report recommending removal in cases of LFS using implants with textured surfaces. BIA-ALCL-derived cell line studies have shown evidence of dysregulation of p53 signaling pathways in response to DNA damage[18)]. Therefore, it is possible that BIA-ALCL develops due to inadequate tumor-suppressor activity in LFS.

Characteristics of the variant in this case

In LFS, missense variants account for about 70% of cases, and the variant in this case is a frameshift variant that has been reported in three cases, two of them developed osteosarcoma, breast cancer and no evidence of onset in childhood (Table 3)[19, 20)]. Genotype-phenotype correlation has been reported for the *TP53* gene, and variable phenotypes, expressivity, and penetrance of cancer are frequent[21)]. Recently, variants leading to loss of function of p53 have been reported to have a more severe phenotype than patients with partial loss[22)]. The loss-of-function form has an early onset of primary cancer, often developing sarcoma or breast cancer by the age of 35 and meeting the classical LFS and Chompret's criteria. The pathological variant in this case has also been reported to show normal p53 protein loss of function. In this case, the frameshift mutation has altered the structure of the p53 protein itself, so it is not detected by immunostaining.

The possibility of a paternal origin has not been completely ruled out, since the LFS has a 75% male penetrance and the paternal uncle is also suspected of having skin cancer. However, since the probability suggests that our case could be a single case, it is necessary to consider the possibility of germline mosaicism, which occurs at a frequency of 2.4% in addition to *de novo*[8)].

Establishing a continuous surveillance system

A lifetime risk of developing cancer, and regular systemic screening is considered important in LFS. Currently, surveillance based on the Toronto protocol has been introduced worldwide for TP53 carriers (Table 4)[13)]. Meanwhile since there are some differences in surveillance subjects and the timing of the start of surveillance subject depending on the region, it is better to refer to the guideline by Kumamoto et al. in Japan[10)]. It is not clear whether the current surveillance studies contribute to improved survival rates due to the short observation period and the low incidence of the disease.

Table 3 Comparison between previous reports and this case with c.216dupC in *TP53* (p.Val73Arg fsX76)

	Togucihda et al 1992	Zerdoumi Y et al 2017	Our case 2021
Medical History	15yF Osteosarcoma	Pt22, 27yF Breast cancer Pt21, 28yM Unaffected	16yF Osteosarcoma 29y Lt-breast cancer 33y Rt-breast cancer
Family history	Mother: 25y Breast cancer	Not listed	Not specific

F, female; M, male; Lt, left; Rt, right; y, years old; Pt, patient.

Table 4 Toronto protocol: agreed surveillance recommendations for *TP53* carriers[13]

ACC	Abdominal US, every 3–4 month: birth–40 y, Biochemistry (17 OH–progesterone, total testosterone, DHEAS, androstenedione) should only be performed where there is an unsatisfactory USS.
Breast cancer	Annual dedicated MRI from age 20–70 years (woman only) Consider risk–reducing mastectomy from age 20 years
Brain tumor	Annual dedicated brain MRI from birth (first MRI with contrast)
Sarcoma	Annual WB–MRI from birth, Abdominal US 3–4 monthly: from 18 y
Colon	Colonoscopy every 2 y: from age 25 or 10 y before earliest onset of colorectal carcinoma in family
Gastric	Recommend Helicobacter pylori testing and eradication if required Endoscopy not indicated due to lack of evidence
Skin	Annual dermatology review from 18 years (general practitioner or dermatology), Japanese is not common. General advice on use of high protection factor sunscreen and covering up in sun.
Other	Recommend detailed discussion of red flag symptoms in both children and adults and provide information on relevant resources. Discuss importance of making positive lifestyle choices (e.g.: not smoking, eating a healthy diet, limiting alcohol consumption, sun protection, keeping physically active and providing appropriate resources).

ACC, adrenocortical carcinoma; DHEAS, dehydroepiandrosterone sulfate; MRI, magnetic resonance imaging; US, ultrasound; WB, whole–body; y years.

However, in a meta–analysis of whole–body MRI surveillance, most of the cancers detected were localized and curative treatment was achieved[23]. Therefore, there is a high possibility of reducing complications and improving quality of life by reducing the intensity of treatment. In addition, some studies have reported that routine examinations can contribute to a reduction in anxiety[24].

Due to the rarity of the disease, the annual surveillance system recommended for LFS is not currently in place nationwide. Although some other facilities perform brain, thoracic, abdominal, and pelvic (including lower limb) imaging four times a year, surveillance that requires frequent visits lacks continuity. Therefore, we proposed a surveillance system that includes whole–body and brain MRI[25], with collaborations between the genetics department, radiology department, breast oncology department and the family clinic.

Conclusion

With the spread of cancer genomic medicine, the diagnosis rate of hereditary tumor syndromes is expected to increase in the future. For these diseases, extra consideration should be given when deciding on a cancer treatment plan and a lifetime surveillance system is necessary. At our hospital, which has a genetic outpatient clinic, we have established a surveillance system including whole–body MRI for LFS, which hopefully will be taken up by other hospitals in Japan.

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Author contributions

RS carried out the acquisition of data and wrote the manuscript. YH and HS conducted pathological assessments. KS and SS conducted whole–body MRI test and assessments. NS and MA reviewed and revised the manuscript. MU, MS, and MA were responsible for the care of the patient. All authors read and approved the final manuscript.

Conflicts of interest statement

None declared.

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Perspectives

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Clinical Research Support Activities in Core Clinical Research Hospitals Throughout Japan

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We evaluated the current status of supporting activities in core clinical research hospitals across Japan using the Japan Registry of Clinical Trials (jRCT) data given the lack of previous research objectively investigated supporting activities for clinical trials in core clinical research hospitals throughout Japan. Briefly, our findings showed that despite the officially supposed cooperation scheme, core clinical research hospitals have not been the primarily selected contractor for clinical research supporters. Considering that core clinical research hospitals are designated spearhead physician-led clinical trials and are responsible for ensuring the quality of such trials, there is a need to determine why the officially supposed cooperative scheme between core and non-core hospitals are still not established in Japan in order to increase the development and quality of Japanese clinical research with maximum efficiency moving forward.

Key words: clinical research support, core clinical research hospital, the Japan Registry of Clinical Trials (jRCT)

Core clinical research hospital

In Japan, the Medical Care Act has established core clinical research hospitals that are designated to play a leading role in physician-led clinical trials. Moreover, they are responsible for the development of clinical research aimed at uncovering novel innovative pharmaceuticals and medical instruments in Japan according to international standards. These hospitals are also mandated to support clinical research developed in other medical institutions[1–2]. The Clinical Research Act, which came into effect on April 1, 2018 in Japan[3], seeks to prevent research misconduct and ensure the reliability of the clinical studies for not only the research subjects but also the general public. As stipulated by the mentioned legislation, the implementation plans and research outlines of clinical trials are required to be registered with the Japan Registry of Clinical Trials (jRCT) database prepared by the Ministry of Health, Labor and Welfare[4]. The clinical trial implementation system includes information on the principal investigator, co-investigators, program manager, study manager, data managers, monitors, biostatisticians, and auditors[5].

Clinical research support activities in core clinical research hospitals

To the best of our knowledge, no previous research has objectively investigated supporting activities for clinical trials in core clinical research hospitals throughout Japan. Therefore, we sought to evaluate the current status of supporting activities in core clinical research hospitals across Japan using jRCT data. Data for a total of 860 hospitals registered in the jRCT from April 1, 2018 to May 31, 2019, including non-core clinical research hospitals, were reviewed, excluding unknown data. We inves-

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tigated the type of institutions from which non-clinical research core hospitals would request clinical research support in conducting clinical trials.

The results of our survey are summarized in Figure 1. The rate of contact requests by non-core clinical research hospitals to core clinical hospitals for each clinical research supporter is as follows: program manager (1.3%, 11/860), study manager (1.3%, 11/860), data managers (1.2%, 11/903), monitors (0.8%, 7/913), biostatisticians (6.0%, 52/860), and auditors (0.7%, 6/865). It has become clear that core clinical research hospitals have not been primarily selected as a contractor for all clinical research supporters, despite the officially supposed cooperation scheme. Aside from auditors, non-clinical research core hospitals requested support from the clinical research support department of their own institutions.

Conclusions

The core clinical research hospital has primarily been designated to lead clinical trials in Japan. It is important for core clinical research hospitals to conduct secure and high-quality clinical research by themselves while also supporting clinical research conducted by neighboring non-core hospitals. We believe that by channeling clinical research conducted throughout Japan through core clinical research hospitals, the quality of clinical research in Japan will improve considerably. Additionally, patients who wish to participate in clinical trials will be guided by the existence of landmarks, namely core clinical research hospitals nationwide. Furthermore, core research hospitals with several on-going high-quality clinical research studies might effectively train and develop numerous young physicians and researchers who want to enrich their experience on clinical research in the near future. As such, there is a need to determine why the officially supposed cooperative scheme between core and non-core hospitals are still not established in Japan in order to increase the development and quality of Japanese clinical research with maximum efficiency moving forward.

We speculate that one of the major causes of the lack of an established cooperation between core and non-core clinical research hospitals is a mismatch between needs and feeds. The core clinical research hospitals have built a system to provide segmented research support, including program managers, study managers, data managers, monitors, biostatisticians, auditors, and clinical research coordinators. However, we infer that researchers from non-core clinical research hospitals would need multifunctional comprehensive support rather than segmented support.

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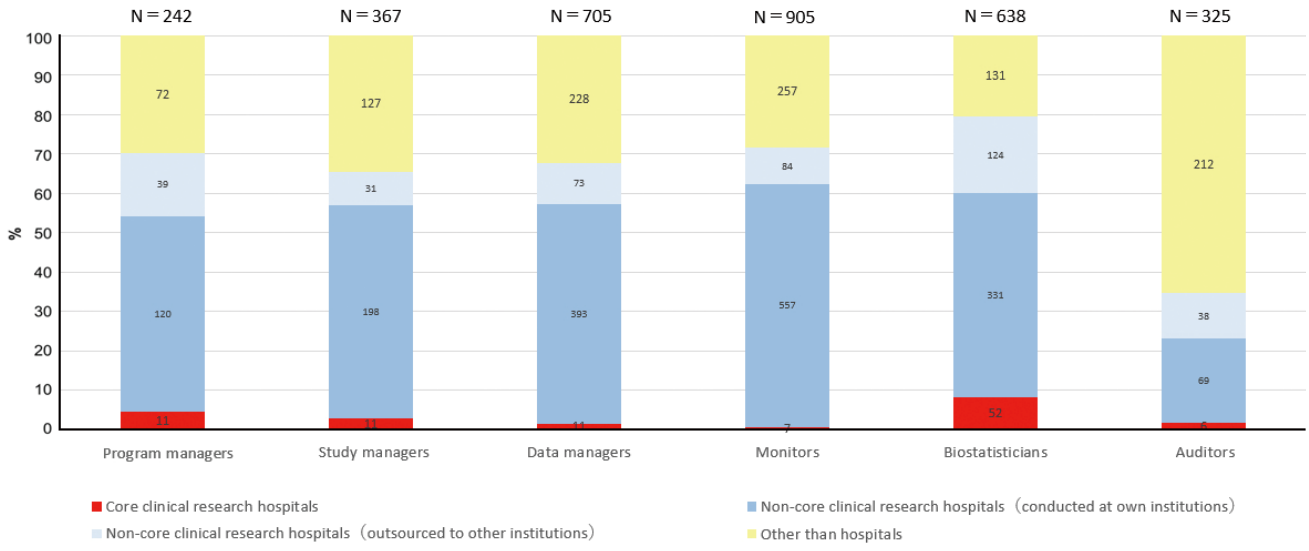

Figure 1 The rate of contact requests by non-core clinical research hospitals and information of outsourcers for clinical research supporters.

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Author contributions

YN and RU have designed this study as a whole and written this manuscript. SN has contributed to statistical analyses. YN, RU, and SS have contributed to data collection. All authors have contributed, provide advice on the interpretation of the results, and approved the final manuscript.

Conflicts of interest statement

There are no conflicts of interest to declare.

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編 集 後 記

68巻4号をお届けいたします。早いもので2022年も下半期に入りました。上半期を振り返ると、世間一般では大きな話題が3つありました。残念ながら暗いニュースばかりです。

まず、本年2月からのロシアのウクライナ侵攻。順天堂大学ではいち早く募金活動を開始し、医学部ではウクライナの医学生や研究者の受け入れも行いました。しかし、本稿を執筆している8月上旬現在、紛争の終結はまだ遠いように思われます。2つめは、7月8日の安倍元首相の銃撃事件。3つめは、COVID-19の第7波。ゴールデンウィーク頃には下火になったかにみえたのですが、7月初旬からの第7波は第6波の約2倍のスピードで感染拡大し、日本全国での一日あたりの新規感染者数は20万人を超えてさらに拡大しつつあります。7月中旬～下旬には本学の医学部生にも感染/濃厚接触による自宅待機者が急増しましたが、第7波はお盆過ぎにはピークアウトとの予測もあるようです。本誌が公開される頃には収束に向かっていることを祈るばかりです。

一方、順天堂大学での明るい話題として、4月から医療科学部がスタートしました。臨床検査技師や臨床工学技士を養成する、第7番目の学部です。

なお、医療科学部のある日の出キャンパスには、再来年度にむけて「薬学部」設置申請の準備が進んでいるようです。

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本誌を通して、先生方の御研究内容の情報共有が学部間で促進され、多職種連携にも貢献できれば幸いです。積極的な投稿をお待ちいたします。

松本　顕
医学部一般教育生物学研究室

イラスト作者より

7月の半ば、実に30年ぶりに奄美大島に行って来ました。久し振りに見るエメラルドグリーンの海や、真白な砂浜、打ち寄せる波の音、そしてアダンの実・・・田中一村（島に移り住んだ日本画家）の絵そのものの世界に浸り、大満足の旅でした。（宮道明子）

順天堂醫事雑誌の記事については既に明治8年の創刊号から電子化されており，J-STAGE（科学技術情報発信・流通総合システム）の電子ジャーナル公開システムにおいて閲覧することができます．順天堂医学会のホームページからもご覧いただけますので，ご活用頂ければ幸いです（https://www.juntendo.ac.jp/journal/）．

特集の企画募集

「順天堂醫事雑誌」では，医学界の最新知識を紹介するために，特集として総説を毎号に掲載しています．
読者の皆様には，特集として相応しい企画等がございましたら，編集室宛にご提案下さいますようお願い申し上げます．

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抄　録

順天堂醫事雑誌
2022. 68(4), 451

不断前進、救命救急　今、ふたたび「仁」

田　中　　裕

順天堂大学医学部附属浦安病院救急災害医学

この度，2022年3月末日をもって定年退職を迎えます．小川秀興理事長をはじめ，これまでご指導，ご支援くださいました多くの方々に心より感謝申し上げます．私は1982年大阪大学を卒業し，特殊救急部に入りました．当時重症外傷や熱傷，中毒，敗血症などの重症患者が連日運ばれてきました．スタッフは現場でスキルを磨き，教え合い，研究に切磋琢磨しました．救命出来ずに多くの悔しい思いもしました．その後，救急医療のニーズも変化し，救急医の診療領域は，病院前救急医療から初期診療，集中治療，災害医療など多岐に及んでいます．2007年9月に順天堂大学浦安病院へ赴任しました．赴任間もなくサッカー全日本監督の緊急入院や，中国冷凍ギョーザ事件で浦安病院が注目を浴びました．救急医療は社会を映す鏡と言われています．私自身，多くの災害や事件などを経験してきました．現在もCOVID-19パンデミックと闘っています．赴任して15年が経ちますが浦安病院の仲間は62名になります．皆救急科専門医など多種の専門医を取得しています．18名が学位を取得し，ハーバード大学やNYフェインスタイン研究所などに多くが留学しています．2019年，第47回日本救急医学会総会を主催しました．本学会のテーマは，「不断前進，救命救急，今ふたたび　仁」です．救急医療は「医」の原点であり，すべての国民が生命保持の最終的な拠り所としています．これからも我々救急医は消えようとしている命の灯を照らし続けるために，最後まで諦めずに患者に寄り添い救命医療を行ってまいります．最後に今まで私を支えてくれた教室員一同，家族に感謝します．順天堂のますますのご発展とお世話になりました全ての方々のご健勝を祈念しまして，定年退職の挨拶とさせて頂きます．

キーワード：救急医療，病院前救急医療，プライマリケア，集中治療，災害医療

この抄録は，順天堂醫事雑誌68巻4号，p324-331，2022掲載の『Fudan Zenshin, Kyumeikyukyu ; ~Now JIN again~』の和文抄録です．

抄　録

順天堂醫事雑誌
2022. 68(4), 452

放射線治療の42年間の発展と放射線治療学講座

笹　井　啓　資[1, 2)]

[1)]順天堂大学医学部放射線治療学講座（放射線腫瘍学・医学物理学）
[2)]美杉会佐藤病院　放射線治療センター

1981 年からの放射線治療の歴史と順天堂大学放射線治療学講座について述べる．1981 年当時，治療計画は X 線透視装置により骨などのランドマークを用いて行っていた．治療も低エネルギー光子を用い，照射範囲のトリミングも鉛ブロックを用いた簡単なものであった．このため，病変部に必要十分な線量を照射できないことがしばしばであった．80 年代後半から 2000 年にかけて放射線治療は革命的に進歩した．コンピュータ技術，精密機械技術，画像診断技術の飛躍的進歩に伴い，コンピュータ制御可能な多分割絞りを装備した超高エネルギーリニアックと CT 画像などを用いた３次元治療計画装置が導入された．この成果として，治療標的に一致した線量分布で放射線を照射することができる強度変調放射線治療や，小さな病変に大線量を集中できる定位照射などが行われるようになった．また，陽子線や炭素線を用いた治療も普及しだした．

2000 年以降は，上述の技術はさらに発展し一般診療として施行できるようになった．また，治療毎に画像を撮影し，病変の位置や他臓器との関連を見ながら治療する画像誘導放射線治療，さらに照射中の動きを把握できるシステムも開発され，正確に標的に照射可能となっている．また，画像診断の進歩により代謝画像を用いた治療計画も導入されている．放射線治療は，現在，がんの根治治療や緩和治療として標準治療となっている．

2000 年当時，放射線医学講座内の小さなグループだった放射線治療は，現在，法人全体で放射線治療医師 11 名，医学物理士 5 名，治療装置 7 台＋ RALS 1 台，順天堂医院での治療症例数約 1000 例となり，最先端の治療を提供できるようになった．代表的な治療成績は，症例数の多い前立腺癌で低，中，高リスクの 7 年 PSA 非再発率はそれぞれ 100%，92.0%，93.2%，乳房温存療法での 10 年乳房内非再発率 95.3 ～ 95.7%，子宮頸癌 5 年全生存は I，II，III，IVA 期 100，84，78，40% である．研究面は長らく低迷していたが，21 年には英文論文数 14 編を達成した．

約 40 年間で放射線治療は革命的な発展をした．これからも，装置の進歩に伴い長足の発展が期待できる．放射線治療学講座は教育，研究，診療面で講座としてようやく形が整ってきた．

キーワード： 放射線治療，放射線腫瘍学，画像誘導放射線治療，強度変調放射線治療

この抄録は，順天堂醫事雑誌 68 巻 4 号，p332–338，2022 掲載の『My 42-year Experience in Radiation Oncology』の和文抄録です．

順天堂医学会 会員の皆様

順 天 堂 医 学 会
会長　服部　信孝

順天堂医学会短期海外留学時助成金給付制度

順天堂医学会では短期海外留学時助成金給付制度を開始いたしました。

1. 要件

下記すべての要件を満たす者

(1) 順天堂大学（大学院を含む）の学生で 1 か月以上 12 か月未満の海外留学をする者

(2) 留学先の研究機関または財団などからの援助がない者

(3) 医学会の正会員として 1 年以上の経歴を有し、医学会費を完納している者

2. 申請書類

(1) 順天堂医学会短期海外留学時助成金申込書

(2) 所属長の推薦書

(3) 申請者の主な研究テーマ・研究業績

(4) 留学受け入れ機関の指導者からの推薦状

3. 助成金の給付金額

留学期間	助成金額
1 か月以上 4 か月未満	10 万円
4 か月以上 7 か月未満	20 万円
7 か月以上 12 か月未満	30 万円

4. 申請スケジュール（年 2 回）

申請期限	助成決定時期
6 月末	8 月
12 月末	2 月

5. 選考機関：順天堂医学会短期海外留学時助成金選考委員会

6. 助成後の義務

(1) 帰国後直近の順天堂医学会学術集会において研究成果の発表および、その内容を「順天堂醫事雑誌」に報告する。

(2) 帰国後は、順天堂大学またはその関連機関に原則として 3 年以上勤務する。

7. 本件の照会先

HP：https://www.juntendo.ac.jp/journal/membership/benefit_plan.html

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