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順天堂醫事雜誌

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(940th issue)

JUNTENDO MEDICAL JOURNAL

順天堂醫事雜誌

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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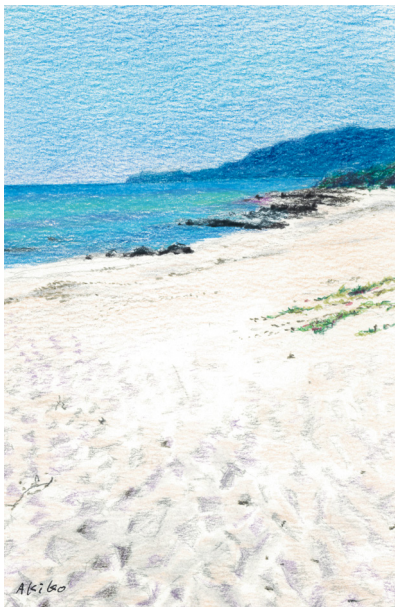

Contents

The Juntendo Medical Society

From the illustrator: As I mentioned in the "From the illustrator" of the previous issue, I went all the way to Amami-Oshima Island, and couldn't help painting the beautiful ocean and the beach of the island. I was fascinated with not only various blue colors of the sea but also the pattern and colors of the sand created by waves and winds (the natural formative art).

Special Reviews

Juntendo Medical Journal
2022. 68(5), 454–458

The Transition of Urological Practice for Forty Years in Juntendo University

YOSHIRO SAKAMOTO

Department of Urology, Juntendo University Nerima Hospital, Tokyo, Japan

I enrolled in the Juntendo University Urological Course in 1982 and worked at Juntendo for 40 years until I retired in 2022. The transition of Urology had been slow until recent years. In the last 20–30 years, Urological practice has made significant progress. I will look back on the 40 years of Juntendo University and describe it in this article. In particular, the transition and breakthrough in the diagnosis and treatment of prostate cancer were remarkable. This point will be described mainly based on the experience at Juntendo Nerima Hospital.

Key words: prostate cancer, transperineal prostate biopsy, robot-assisted radical prostatectomy

In Japan, in the early Meiji era, German medicine was required to have the norms of modern medicine, and Urology was introduced along with Dermatology in the early 30th year of the Meiji era. Therefore, Urology in Japan has a strong connection with Dermatology until after World War II, and the course has continued as a Dermatology and Urology class at each medical education institution. On the other hand, in the United States, the Urology was the department of surgery, division of Urology. At the dawn of Juntendo University, the Department of Urology discharged very talented and well-known urologists.

Juntendo Urology Faculty Part-1

1901 (Meiji 34) Faculty of Juntendo: Susumu Sato (Surgery), Tatsujiro Sato (Surgery), Saburo Akutsu (Urology)

Prof. Saburo Akutsu (Figure 1) studied Urology at the University of Vienna and was one of the pioneers of Japanese Urology.

1908 (Meiji 41) Prof. Isamu Sakaguchi (Figure 2) opened Dermatology.

1915 (Taisho 4) Dr. Sakaguchi became a professor of Dermatology and Urology, and later became a full-time in Urology. He made an effort in the domestic production of cystoscopes. In recognition of his achievements, the Sakaguchi Award was established at the Japanese Urological Association.

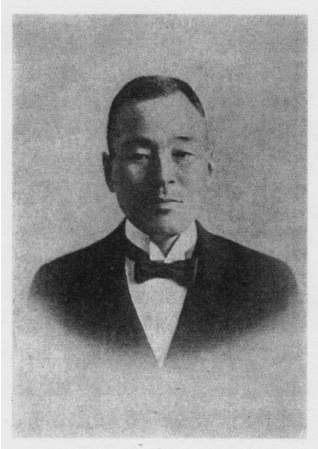

Figure 1 Prof. Saburo Akutsu

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Figure 2 Prof. Isamu Sakaguchi

Juntendo Urology Faculty Part-2

1943 (Showa 18) Prof. Masatoki Koike
1958 (Showa 33) Prof. HiromotoTakahashi
1982 (Showa 57) Prof. Ryuichi Kitagawa
1993 (Heisei 5) Prof. Makoto Fujime
2012 (Heisei 24) Prof. Shigeo Horie

The Transition of Urology for forty years

The prostate cancer practice has undergone significant changes over the last 40 years. Forty years ago, when I was still a new physician, surgical castration was the first operation for a new urologist. At that time, surgical castration was the only surgery for prostate cancer and was the mainstay of hormone therapy for prostate cancer. Currently, robot-assisted radical prostatectomy (RARP, Figure 3) has become a main surgical procedure that is widely performed not only in Western countries but also in Japan. Hormone therapy for prostate cancer has also started to use new AR target drugs (Abiraterone, Enzalutamide, Apalutamide, and Darolutamide) as well as surgical castration and medical castration. In addition, anticancer drugs such as Docetaxel and Cabazitaxel have been used, and radiation therapy for bone metastases such as Ra-223 has also been administered.

Prostate biopsy has also changed significantly. In the 1980s, when induration of prostate cancer was palpable, prostate biopsy was performed using a TruCut needle (Figure 4) under a finger guidance. In recent years, puncture needles have also advanced, allowing us to use superior products (Figure 5). PSA measurement became possible by Wang et al.[1] in 1979, Holm et al.[2] performed a transrectal ultrasound-guided prostate biopsy (TRUS-B) in 1981.

In Japan, in 1963, Hiroki Watanabe of the Department of Urology, Kyoto Prefectural University of

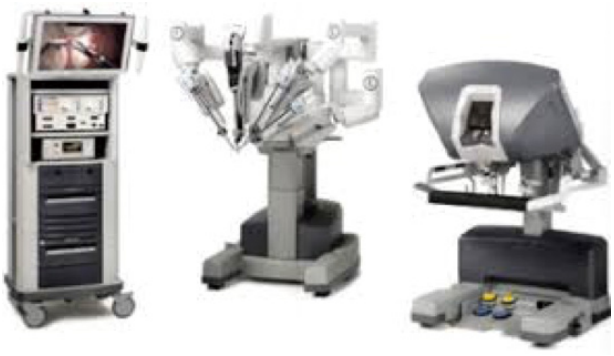

Figure 3 Intuitive Da Vinci surgical system

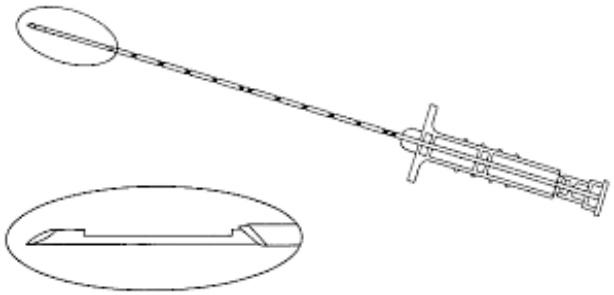

Figure 4 TruCut needle

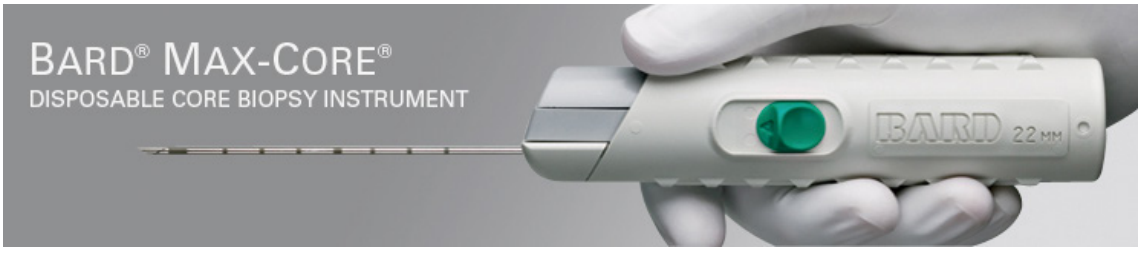

Figure 5 BARD® MAX-CORE®

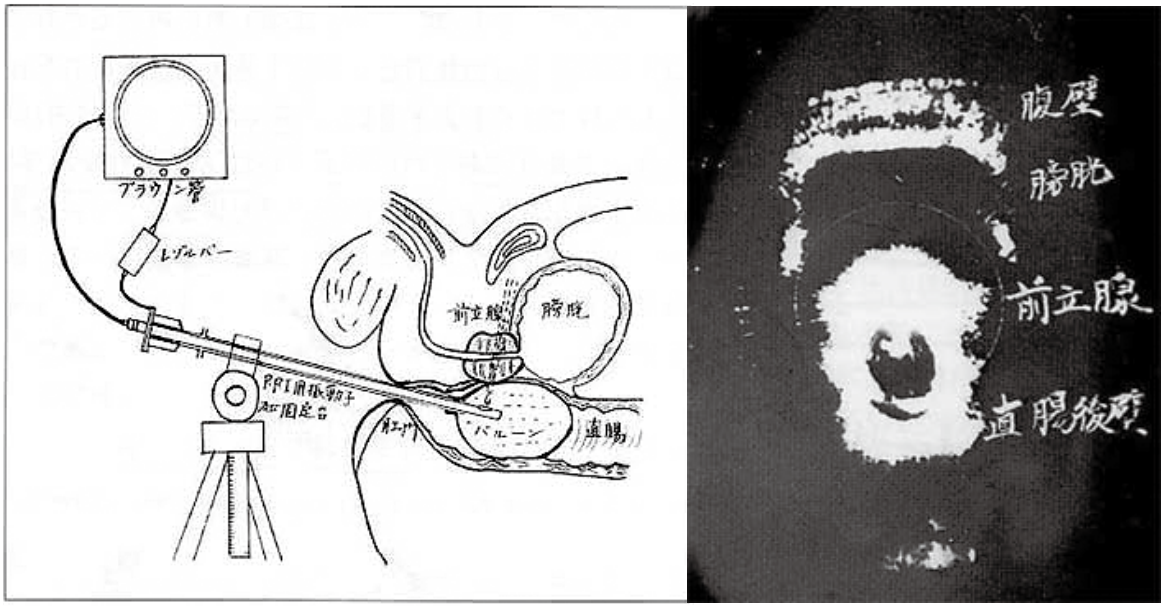

Figure 6 Transrectal prostate ultrasound

Medicine, Tatsuo Ouchi, Hiromoto Takahashi, and Toshio Wagai of Juntendo University developed and clinically applied transrectal prostate ultrasound (Figure 6).

Analysis of prostate cancer localization toward improved diagnostic accuracy of transperineal prostate biopsy[3]. Delineating the precise localization of prostate cancer is important in improving the diagnostic accuracy of prostate biopsy. In Juntendo University Nerima Hospital, initial 12-core or repeat 16-core biopsies were performed using a transrectal ultrasound guided transperineal prostate biopsy method. We step-sectioned prostates from radical prostatectomy specimens at 5-mm intervals from the urethra to the urinary bladder and designated five regions: the (1) Apex, (2) Apex-Mid, (3) Mid, (4) Mid-Base, and (5) Base. We then mapped prostate cancer localization on eight zones around the urethra for each of those regions. Prostate cancer was detected in 93 cases of 121 cases (76.9%) in the Apex, in 115 cases (95.0%) in the Apex-Mid, in 101 cases (83.5%) in the Mid, in 71 cases (58.7%) in the Mid-Base, and in 23 cases (19.0%) in the Base. In 99.2% of all cases, prostate cancers were detected from the Apex to Mid regions. For this reason, transperineal prostate biopsies have routinely been prioritized in the Apex, Apex-Mid, and Mid regions, while the Base region of the prostate was considered to be of lesser importance. Our analyses of prostate cancer localization revealed a higher rate of cancer in the posterior portion of the Apex, antero-medial and postero-medial portion of the Apex-Mid and antero-medial and postero-lateral portion of the Mid. The transperineal prostate biopsies in our institute performed had a sensitivity of 70.9%, a specificity of 96.6%, a positive predictive value (PPV) of 92.2% and a negative predictive value (NPV) of 85.5% (Figure 7, 8, Table 1, 2).

Conclusions

The concordance of prostate cancer between prostatectomy specimens and biopsies is comparatively favorable. According to our study, the diagnostic accuracy of transperineal prostate biopsy

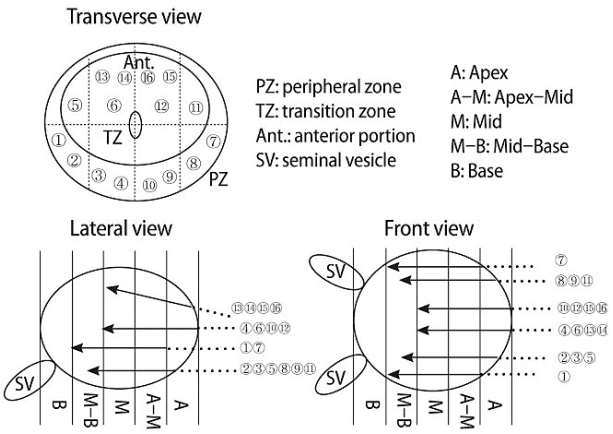

Figure 7 Transperineal prostate biopsy : transverse view, lateral view, and front view

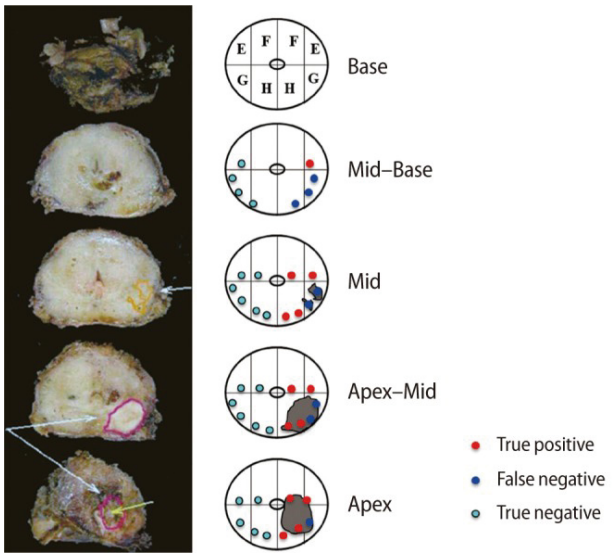

Figure 8 Transverse view : prostate cancer localization and transperineal prostate biopsy

Table 1 Prostate cancer localization in the Apex, Apex–Mid, Mid, Mid–Base, and Base out of the 121 cases

Region	No. of cases of prostate cancer positive (%)
Apex	93 (76.9)
Apex–Mid	115 (95.0)
Mid	101 (83.5)
Mid–Base	71 (58.7)
Base	23 (19.0)

Ninety-nine point two percent of all cancer cases were detected in the Apex and Apex–Mid regions, 95.5% was detected in the Apex–Mid and Mid regions, and 84.3% was detected in the Mid and Mid–Base regions.

Table 2 Concordance of prostate cancer in prostatectomy specimen and biopsy

	Prostate cancer (+)	Prostate cancer (−)	Total
Biopsy (+)	344	29	373
Biopsy (−)	141	834	975
Total	485	863	1,348

Sensitivity (=TP/[TP+FN]), 70.9%; specificity (=TN/[FP+TN]), 96.6%; positive predictive value (=TP/[TP+FP]), 92.2%; negative predictive value (=TN/[TN+FN]), 85.5%.
True positive (TP) False positive (FP)
False negative (FN) True negative (TN)

Prof. Ryuichi Kitagawa
(Died in 2020)

Prof. Makoto Fujime

Prof. Shigeo Horie

Emeritus Director Takeshi Miyano

Director Kuniaki Kojima

Figure 9 Three chief professors and two directors who were my mentors

can be improved in our institute by including the anterior portion of the Apex–Mid and Mid regions in the 12–core biopsy or 16–core biopsy, such that a 4–core biopsy of the anterior portion is included.

During my 40 years at Juntendo University, I served three chief professors and two directors (Figure 9). Thanks to these doctors, I was able to carry out a very fulfilling and happy medical practice. We would like to express our sincere gratitude to all the bosses and staff who have been involved in reaching retirement age.

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Author contributions

YS contributed to drafting the manuscript, figures and tables.

Conflicts of interest statement

The author declares that there are no conflicts of interest.

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Special Reviews

Juntendo Medical Journal
2022. 68(5), 459–464

Historical Review of Stereotactic Radiosurgery in Juntendo University

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Department of Radiation Oncology Juntendo University Nerima Hospital, Tokyo, Japan

Juntendo University Hospital is the second hospital in Japan to start stereotactic brain irradiation using linear accelerator (LINAC) system. This report details the historical transition of stereotactic irradiation, progress of treatment technology, and change of treatment method from the beginning to the Juntendo University Hospital and Juntendo Nerima Hospital. The hospital changed the use of cobalt to the LINAC system when it was rebuilt in 1993. Total body irradiation treatment for leukemia had started around the same time. A year later, in 1994, the hospital used their LINAC systems to perform stereotactic head irradiation, otherwise known as pinpoint irradiation. In 2005, Juntendo University Nerima Hospital was opened and in September of the same year, radiation therapy using the latest model of LINAC system at that time was initiated. This was the first among all Juntendo hospitals to start intensity-modulated radiation therapy (IMRT) and image-guided radiotherapy (IGRT). In 2014, a second LINAC system for IMRT and IGRT was equipped at the Juntendo Hongo Hospital. In 2021, the LINAC systems of the Juntendo University Nerima Hospital were replaced after 15 years of usage. The new method of SRS was started using a latest LINAC systems.

In this paper, I introduce the technique and progress of SRS that I have experienced mainly in Juntendo University.

Key words: stereotactic radiosurgery, stereotactic radiotherapy, linear accelerator, Gamma Knife

Introduction

The Japan Radiological Society defines single stereotactic irradiation as stereotactic radiosurgery (SRS) and fractionated stereotactic irradiations as stereotactic radiotherapy (SRT), but in this manuscript, the term SRS is used without distinction. In Japan, SRS using the Gamma Knife was first started at the Tokyo University Hospital in 1991. Two years later, SRS using linear accelerator (LINAC) systems was performed at the Nagasaki University. A year later, in 1994, the Juntendo University Hospital began SRS using the LINAC systems, making it the second facility in Japan to perform LINAC-based SRS. Since then, the LINAC-based treatment method has progressed along with the technological development of the LINAC systems. I introduce the historical background and the development of SRS technique especially for Juntendo University Hospitals.

Short history of Stereotactic Radiosurgery for brain in Japan

In 1968, the world's first SRS was started with the Gamma Knife technology at the Karolinska Hospital in Sweden (Table 1). Twenty-three years later, the first Gamma Knife surgery in Japan was started at the University of Tokyo. The successful adoption of the LINAC system for SRS by the Juntendo University, Japan, led to a rapid development of LINAC devices and subsequent increase in the number of treatment facilities in Japan.

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Table 1 Short history of brain Stereotactic Radiosurgery in Japan

1968	Gamma Knife was bone in Karolinska Sweden
1991	The first Gamma Knife Unit was started at Tokyo University in Japan.
1993	The first Linac Radiosurgery system in Japan was started in Nagasaki Univ Hosp.
1994	The second Linac Radiosurgery was started in Juntendo Univ Hosp.
1997	Cyber knife treatment started in Japan.
1999	Mitubishi Electric co. released the C-armed Radiosurgery system.
2000	IMRT started in Japan
2004	Linac Radiosurgery covered by insurance in Japan. (63000)
2009	Linac radiosurgery with micro-multileaf collimator by Elekta synergy started at the JSDF Hosp.
2014	Gamma Knife I-con released. (Automatic Mask Syetem)
2014	Lancet oncology 2014: Stereotactic radiosurgery for patients with multiple brain metastases (JLGK0901 : Stereotactic radiosurgery without whole brain radiotherapy as the initial treatment for patients with five to ten brain metastases is non-inferior to that for patients with two to four brain metastases in terms of overall survival.
2020	Linac Radiosurgery for multiple brain metastasis started by Elekta Versa HD

In 2009, the Self-Defense Forces Central Hospital, Japan, routinely performed SRS with a micro multi-leaf and a mask system. Prior to that, SRS was performed by fixing the patient's head with an invasive metal pin. A non-invasive method of fixation was desirable to reduce the anxiety of the patients. In 2014, the Gamma Knife technology developed a mask system. The same year, a multicenter collaborative study reported positive outcomes of the Gamma Knife treatment for multiple brain metastases[1]. Following this, the Gamma Knife treatment was actively performed in cases of multiple brain metastases to preserve the cognitive function. The history of SRS in Japan began with the Gamma Knife technology. A few years later, this was replaced by the LINAC system owing to the rapid technological development of both the software and hardware, which continues to the present date.

The first case of SRS in Juntendo University

The first LINAC system was equipped at the Juntendo University in 1993. Figure 1 shows the Winston-Lutz test[2] that performs quality control of couch and gantry less than 2 mm. Figure 2 illustrates the star-shot technique. Using a 2 mm collimator, a pencil beam is emitted from the LINAC system to the low-sensitivity X-ray film. This

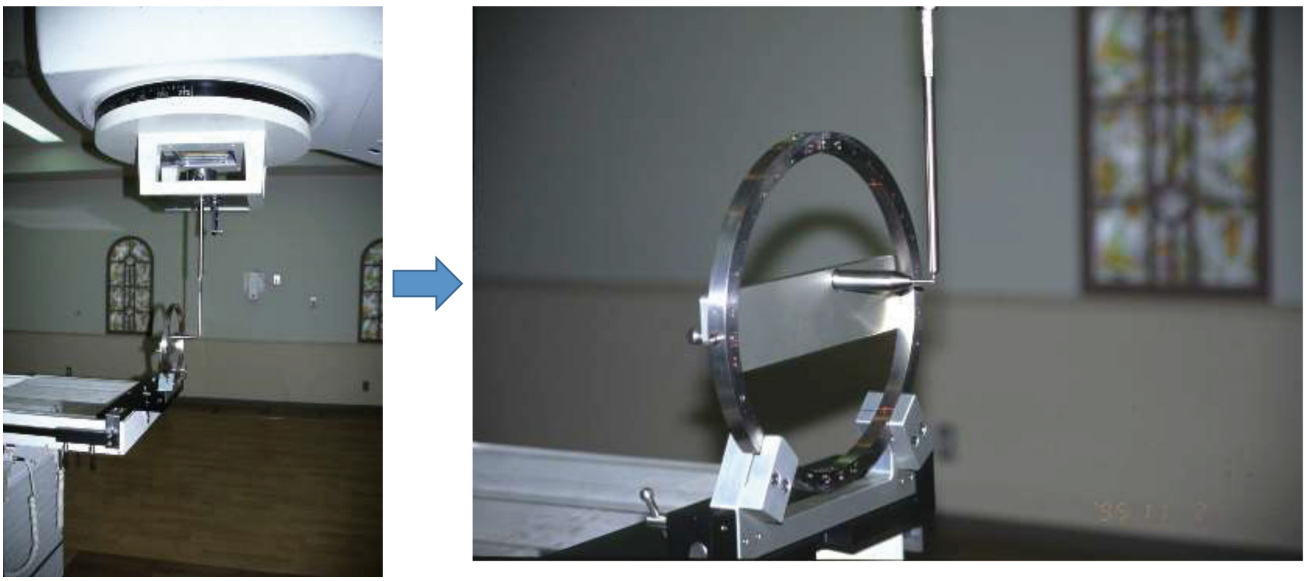

Figure 1 Winston-Lutz test of the first linear accelerator installed in Juntendo University

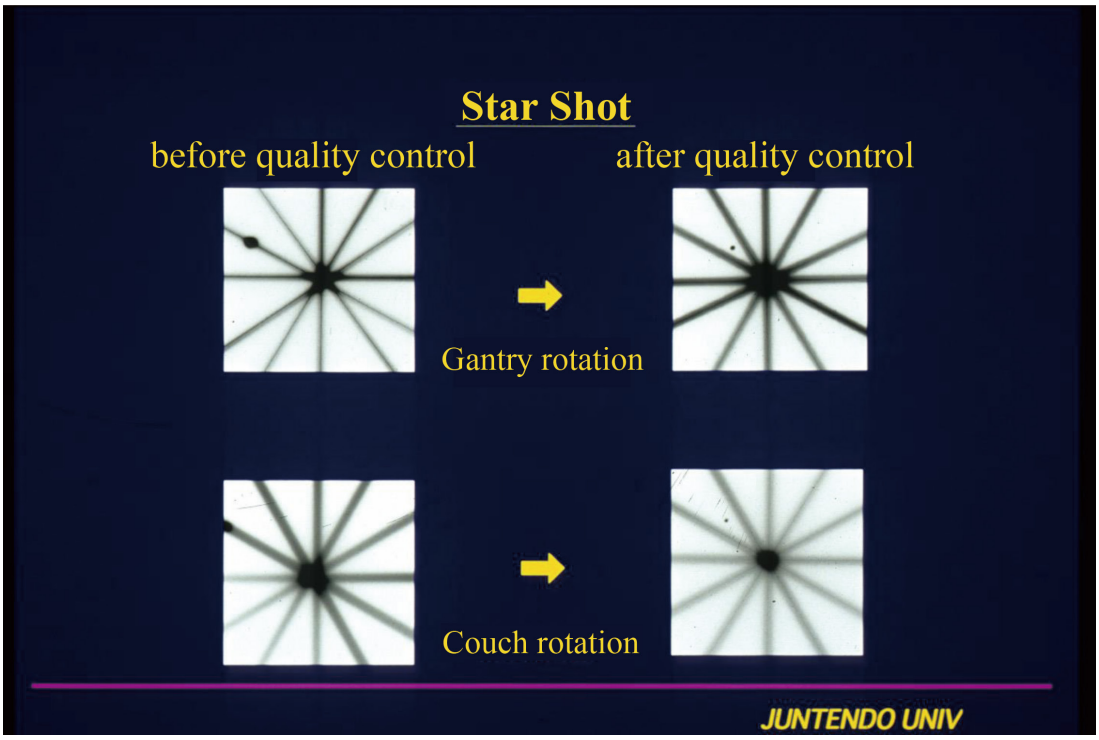

Figure 2 Star shot before and after quality control

enables accurate confirmation of the isocenters of the gantry and couch. Considerable amount of time and effort were required for the quality control step, as 30 years ago, the accuracy of the LINAC system was suboptimal. Once the quality control procedures were complete, the SRS was started. The first case of SRS in Juntendo University involved a patient with cerebral arteriovenous malformation (AVM). The nidus position was analyzed by fusing the images from enhanced computed tomography (CT) and angiography. SRS was performed with five to six directions non-coplanar stereotactic multiple arc radiotherapy. Figure 3 shows a tungsten collimator for converting X-rays to the pencil beam, which enabled the selection of an irradiation diameter from 5 mm to several centimeters according to the size of the target. After attaching the collimator to the LINAC system, the focused pencil beam X-rays irradiated the target. Two years after the SRS, the nidus had completely disappeared without complications (Figure 4).

At that time, many cases of AVM were treated by SRS in Juntendo University. 30 cases of AVM were carried out in two and a half years. These cases were reported in the journal of the Japanese Society of Radiation Oncology. Of the 14 summa-

Figure 3 Tungsten collimator and attachment of Linac system for pencil beam

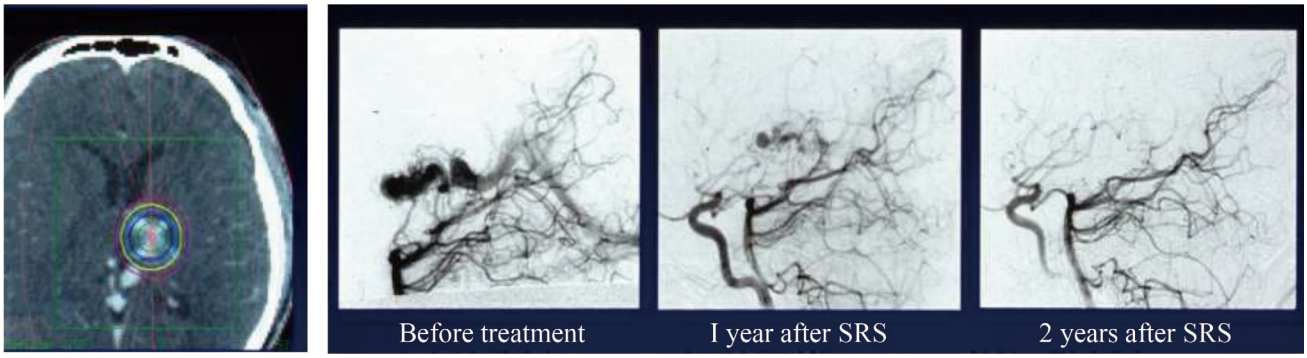

Figure 4 Dose distribution of cerebral AVM and post-SRS images of angiography

rized cases that followed more than 2 years, desirable results were obtained with a nidus obstruction rate of 25% in 1 year and 58% in 2 years and a nidus reduction rate of 91% in 2 years[3]. There were no neurological side effects. Furthermore, in a report on AVM, we investigated the relationship between stagnation time of the contrast medium in angiography and nidus occlusion rate. In this study, angiographs from 25 patients with AVM were analyzed. The results showed that an AVM with a longer stagnation time of the contrast medium was more likely to be occluded[4]. We also summarized the most common cases of brain metastasis that were treated by radiosurgery[5]. At that time, since there was only one magnetic resonance imaging device in Juntendo University, the radiation effect of SRS was evaluated by CT images.

SRS with non-invasive fixation of the head with a mask system

SRS with mask system started at 2009 while I was in the Japan Self-Defense Forces Central Hospital (Figure 5). Because of several trials, we started SRS with a non-invasive mask system

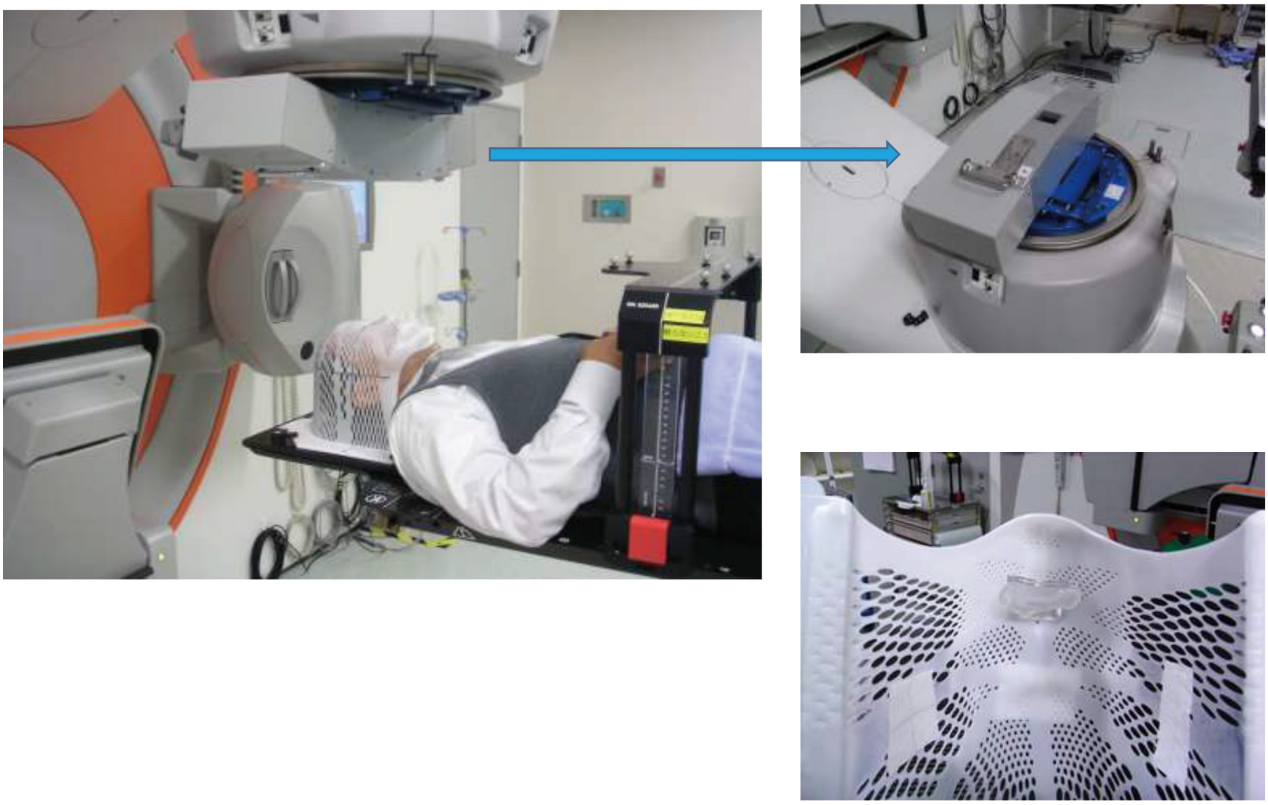

Figure 5 SRS with non-invasive fixation with a mask system

instead of an invasive fixation using a head pin. The LINAC systems used the Elekta Synergy platform (Elekta AB, Stockholm, Sweden), with IGRT, 6-degree robotic couch (HexaPOD)[6], and 3 mm micro multi-leaf to maintain the fixation accuracy below 1 mm. HexaPOD (Elekta AB, Stockholm, Sweden) is a couch that can adjust the twisting element in addition to the XYZ position, and is a system with higher accuracy than the 3-axis couch. SRS using HexaPOD was started for the first time in Japan. Many cases have been treated with acceptable accuracy using this system[7].

SRS with the latest LINAC systems

In general, 80% of SRS cases comprise brain metastases. Until around 2014, whole-brain irradiation was indicated for three to four or more brain metastases. However, in recent years, the advent of molecular targeted drugs has enabled long-term survival of patients even in advanced cancer stages. In such an era background, in recent years, cognitive decline as an adverse effect of whole-brain irradiation has become a problem.

Furthermore, the results of a multicenter joint study by the Gamma Knife group[1] have offered a choice to perform SRS on patients with multiple brain metastases. In 2021, the LINAC systems of the Juntendo Nerima Hospital were updated to the latest model called Versa HD (Elekta AB,Stockholm, Sweden) (Figure 6). Versa HD is a highly versatile Linac capable of stereotactic radio surgery (SRS) for multiple brain tumors and high-definition volumetric modulated arc therapy (VMAT)[8]. It is possible to obtain not only online marker-less 4D-Cone Beam CT (CBCT), but also 3D / 4D CBCT during treatment. Moreover, the treatment time is shorter than that of conventional SRS. Furthermore, the treatment accuracy has an error of 1 mm or less by using a quality control system called Catalyst (C-RAD Positioning AB, Uppsala, Sweden). Although it has become possible to perform SRS for multiple brain metastases using this systems, it is necessary to consider the indication of SRS based on the patient background. An example of SRS for multiple brain metastases is presented in Figure 7. Since this patient had a history of prophylactic whole-brain irradiation due to small-cell lung cancer, it was impossible to re-radiate the whole brain. Eleven brain metastases were effectively treated using this system by concentrating the dose distribution at each of the metastases. The average dose in the normal brain was as small as 8 Gy.

Figure 6 New Linac systems in Juntendo Nerima Hospital (Versa HD by Elekta corp

Conclusion

SRS in Japan began with the Gamma Knife technology, but a few years later, LINAC-based SRS was started. The LINAC-based SRS was widely used, as IMRT, SRS, and conventional irradiation could be performed using the same LINAC system. In recent years, stereotactic irradiation has be selected even for multiple brain metastases if it possible. Although indications of SRS have expanded, methods of radiation therapy in future would need to be selected according to individual patient backgrounds.

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Author contributions

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

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The Author declares that there are no conflicts of interest.

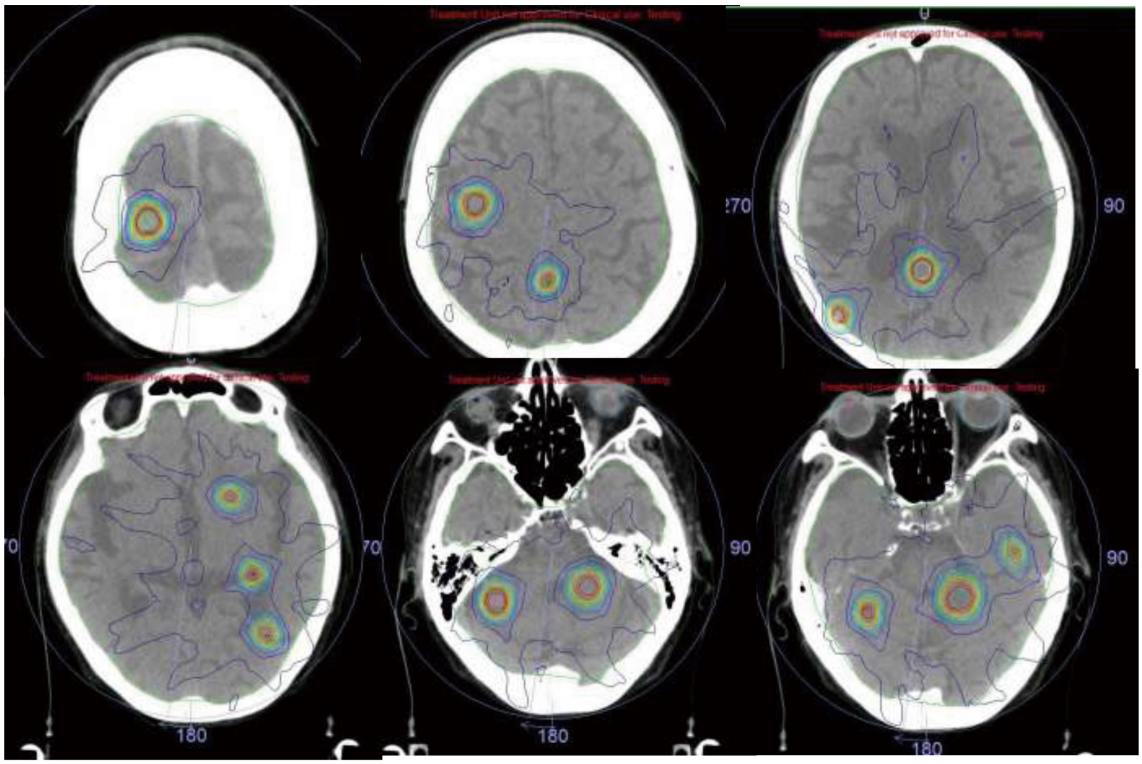

Figure 7 Dose distributions of SRS for 11 brain metastasis using new Linac in Juntendo Nerima Hospital

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Special Reviews

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New Countermeasures Against Infections with/after COVID-19: Is Chlorine Dioxide a Useful and Safe Disinfectant?

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Pandemics of microorganism are serious problem such as corona virus induced disease 2019(COVID-19), and the infectious diseases rapidly transmitted via airborne or aerosol among community space. To prevent aerosol infections, ozone and chlorine dioxide gases are practical methods in room air. However, ozone requires relatively high concentrations for this purpose, which might be toxic to humans present in the room. On the other hand, the low concentration of chlorine dioxide gas and aqueous solution are sufficiently effective against aerosol infection for the causative microorganism, and it is expected that when it is used in combination with a high-efficiency particulate air filter, it will be highly safe with high prevention effect and cost effectiveness.

Key words: chlorine dioxide, COVID-19, aerosol infection, disinfection

Introduction

Science the current global outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the development of effective and safe methods to inactivate viruses in a manned environment have been required[1].

Respiratory viruses mainly transmitted human-to-human via droplets or aerosols. To control infections, not only standard precaution but also countermeasures at the spatial environment are important, and the establishment of economically healthy and effective infection prevention methods is desired.

Disinfection methods

Ozone

Ozone gas is a strong oxidant[2] and has long been used to inactivate pathogenic microbes in water[3,4]. It can be used to inactivate microbes on the surfaces of objects and aerosols. Hudson et al. found ozone inactivated viruses, including mouse coronavirus, on glass and stainless steel[5]. They reported that 20–25 ppmv ozone with 90% relative humidity was effective at inactivating 12 viruses on hard or porous surfaces[5].

However, high concentrations of ozone need for the disinfection of room air or object surfaces, and problem remains its toxicity to humans and animals[6]. Sokolowska et al. reported a mouse experiment where a single exposure to 1 ppmv ozone for 60 min caused damage to the bronchiolar epithelium within 2 hours, disrupted epithelial tight junctions, and promoted cell death, which was followed by reactive oxygen species production[6]. This result indicates the difficulty of using ozone as a disinfectant against viruses in the presence of humans in rooms. Chronic exposure of humans to ozone causes the progressive and irreversible loss of alveolar

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epithelial cells and eventually emphysema occurs[7]. Therefore, although the effectiveness of ozone has been proven, its use, especially in terms of concentrations and exposure periods, should be carefully controlled to avoid adverse effects.

Chlorine dioxide

Chlorine dioxide inactivates many viruses. For instance, the inactivation activity of chlorine dioxide against feline calicivirus and influenza A virus was 0.05 ppmv for 5 h, and the reduction of virus was to a level of 10^{-5} [8]. Inactivation of viruses by chlorine dioxide aqueous solution was also demonstrated. Sanekata et al. found that human influenza virus, measles virus, canine distemper virus, human herpes virus, human and canine adenoviruses, and canine parvovirus were inactivated by chlorine dioxide aqueous solution[9]. Hepatitis A virus was completely inactivated after 10 min in a 7.5 mg/L aqueous solution of chlorine dioxide[10]. The cause of inactivation of the virus was related to the complete loss of antigenicity of the virus and the loss of the 5' non-translated region of its genome[10, 11]. Chlorine dioxide is a relatively stable free radical[12, 13] that denatures proteins by oxidizing their tyrosine and tryptophan residues[14-16]. Ogata et al demonstrated that chlorine dioxide is effective against the SARS-CoV-2 and the mechanisms of action that inhibits the binding of the spike protein of the SARS-CoV-2 on ACE2 through the action of chlorine dioxide has been verified[17].

Akamatsu et al. demonstrated the safety of a low concentration of chlorine dioxide in an animal experiment. Rats exposed to whole-body inhalation of 0.1 ppmv chlorine dioxide for six months with a two-week recovery period showed no differences in body weight gain, food intake, water intake, relative organ weight, blood biochemistry data, and hematology examination data compared with control rats not exposed to chlorine dioxide[18]. In their experiment, rats were exposed to chlorine dioxide for 24 hours/day and 7 days/week. Furthermore, the concentration of gas was precisely controlled within ± 25% of the target concentration[18]. Their result strongly suggests that chlorine dioxide at or below 0.1 ppmv can be used safely to disinfect room air in the presence of humans for a long period. A concentration of 0.1 ppmv chlorine dioxide was effective at inactivating virus in room air[19-21]. The US Department of Labor of the Occupational Safety and Health Administration (OSHA) stated that the permissible exposure concentration of chlorine dioxide for humans for an 8-hour time-weighted average was 0.1 ppmv[22]. Dalhamn reported a no-observed adverse-effect-level (NOAEL) of 0.1 ppmv in rats exposed to chlorine dioxide for 5 hours/day for 10 weeks[23]. Therefore, chlorine dioxide can be used effectively and safely at relatively low concentrations against viruses and bacteria.

Ultraviolet (UV)

UV light and photochemical reactions have been used to inactivate viruses. Regarding UV light irradiation, 254 nm light is usually used[24, 25]. While this method is quite useful for inactivating viruses floating in room air or stuck on objects in a room, its disadvantages include that it cannot inactivate virus in the blind spots of a room where UV light does not penetrate. Furthermore, humans cannot be present because UV irradiation causes cataracts[26] and dermal neoplasms[27]. There are other similar methods that can inactivate viruses. For instance, viruses are inactivated by photochemical reactions using titanium oxide[28]. However, this method is useful predominantly to inactivate viruses stuck on objects. Furthermore, it has not been proven to be effective at inactivating virus floating in room air or away from object surfaces.

Hypochlorous acid (HClO)

Aerosol sprays are also used to disinfect room air containing pathological viruses. For this purpose, hypochlorous acid in water is frequently used as a spray to inactivate avian influenza virus, Newcastle disease virus, and coronavirus[29, 30]. An aqueous solution of sodium hypochlorite (NaClO) was also used as a spray to disinfect viruses[31, 32]. However, both hypochlorous acid and sodium hypochlorite solution sprays are primarily used to disinfect viruses on the surfaces of objects. While useful, they are rarely used to disinfect room air, and their effectiveness at inactivating viruses floating in room air has not been demonstrated quantitatively.

Ventilation

The mechanical or natural ventilation of room air is a simple, effective, and inexpensive way to minimize the airborne transmission of respiratory

viruses[33]. The Center for Disease Control and Prevention recommends a ventilation of 6–15 room air changes per hour to minimize the transmission of microbes[33]. However, the efficient ventilation of room air is accompanied by unwanted warming or cooling of room air unless an ambient temperature is appropriate. This requires extra energy expenditure aside from that required for mechanical ventilation, and such procedures go against the earth-warming policies of many countries. Thus, the safe and effective inactivation of viruses in room air by methods other than ventilation should consider energy saving. In summary, after a survey of the current literature, low-concentration chlorine dioxide is the most suitable agent for the safe and effective.

Discussion

Ozone gas is an effective disinfectant in the air. However, its toxicity becomes a serious problem when it is used continuously in a room[5–7]. On the other hand, chlorine dioxide gas can be used in a room with low concentrations that are effective in inactivating viruses and which are safe to humans[18]. Chlorine dioxide gas is the only practical and currently available disinfection agent that can disinfect viruses in room air to prevent viral respiratory diseases[34].

Since ethical support is required when using chlorine dioxide gas in a manned environment, there was a need to obtain approval from the ethics committee of hospital and institution. We installed and applied in an actual hospital environment after careful consideration and discussion by the committee and their approval. After that chlorine dioxide gas generating gel was installed in each room of the pediatric ward in a city hospital during the winter months when infectious gastroenteritis was prevalent, and we were able to conduct a study on the effect of preventing secondary infections of the infectious gastroenteritis using an aqueous solution of chlorine dioxide. As a result of examination of the four seasons since 2016, it was confirmed that there were no secondary infections of infectious gastroenteritis observed and that there were no reported adverse events. (Figure 1)

Nosocomial pneumonia develops in patient around 48 hours after admission. This form of pneumonia has a high mortality rate and incidence rate and is a major problem in terms of the medical expenses it incurs for patients. One of the preventive measures for nosocomial pneumonia includes environmental improvement such as disinfection and cleaning of hospital rooms. However, in many ways, this is an uphill battle, as many infections are transmitted through droplets in the air (as we have seen with the recent COVID-19 pandemic), which are extremely difficult to combat, especially when the main preventive method is cleaning surfaces.

In nosocomial infections, contact infections such as drug resistant bacteria and viruses become a problem. Furthermore, measures such as ventilation of air-conditioning and prevention of aerosol spread by shielding are being taken as measures against aerosol infection and air infection. Standard precaution measures alone leave viruses and bacteria in the environment. Secondary infections also occur in actual medical settings, making it difficult for clusters to occur. It is possible to further reduce the risk of nosocomial infections by adding more reliable infection countermeasure in spatial disinfection and virus removal using chlorine dioxide gas to the conventional infection countermeasures. And the causative microorganisms include not only general bacteria but also drug-resistant bacteria, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Multi-Drug Resistant *Pseudomonas aeruginosa* (MDRPA). (Figure 2)

Of course, measures already exist within hospitals to prevent or counteract air and droplet infections. One of the chief methods is the use of high-efficiency particulate air (HEPA) filters, but even if the pathogenic microorganisms are captured by the filter, they are not sterilized or killed – meaning that the problems are not really solved in any meaningful way. There are also problems with the associated costs of HEPA filters, as well as the issues they cause regarding the need for secure spaces in hospitals – something difficult to achieve when HEPA filters are installed.

On the other hand, chlorine dioxide exists as a gas at room temperature and dissolves in water to form an aqueous solution. Chlorine dioxide gas and aqueous solution have a sufficient effect on aerosol infection of the causative microorganism for nosocomial pneumonia and an improvement of its efficacy can be expected by using it in combination with the conventionally used HEPA filter.

Impact Objectives

- Clarify the route of infection transmission by screening for drug-resistant bacteria and genetically testing pathogenic microorganism
- Identify effective countermeasures for each route of infection

Chlorine dioxide combats infections

***Professor Kaoru Obinata** leads a project aiming to reduce the risk of nosocomial infections through the development of reliable infection countermeasures*

What issues are you trying to solve with your research?

Nosocomial pneumonia is a form of pneumonia that develops 48 hours after admission and the causative microorganisms include not only general bacteria but also drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas aeruginosa* (MDRPA). Nosocomial pneumonia has a high mortality rate and incidence rate, and is a major problem in terms of the medical expenses it incurs for patients. One of the preventive measures for nosocomial pneumonia includes environmental improvement such as disinfection and cleaning of hospital rooms. However, control of infection through the spatial environment is also important, and the establishment of economically healthy and effective infection prevention measures is desired. That is where our work comes in. By screening for drug-resistant bacteria such as MRSA and MDRPA – as well as genetically testing pathogenic microorganism using multiplex polymerase chain reactions (PCR), we can clarify the route of infection transmission and take effective countermeasures for each route of infection. Our team at the Department of Pediatrics, Juntendo University in Japan is focused on doing just this with the aim of helping many patients across Japan and beyond.

From your perspective, what is the ultimate impact of your research?

In this research, in order to examine the efficacy and safety of chlorine dioxide on aerosol infection and contact infection, we will regularly conduct active surveillance for patients admitted in high care rooms or ward. There may be negative and skeptical opinions in the medical field because there is no widespread awareness of the safety and efficacy of low-concentration chlorine dioxide. So, by confirming the preventive efficacy and safety of chlorine dioxide against nosocomial pneumonia, we can expect the establishment of a new nosocomial infection control method with less human and economic burden. Ultimately, it will be beneficial not only for the inpatients but also for the hospitals.

Can you talk about the challenges you have faced in your research?

Since ethical support is required when using chlorine dioxide gas in a manned environment, there was a need for us to obtain approval from the ethics committee of each hospital and institution. It can only be installed and applied in an actual hospital environment after careful consideration and discussion by the committee and their approval.

Have you had any results that you are particularly pleased with?

Chlorine dioxide gas generating gel was installed in each room of the pediatric ward in a city hospital during the winter months when infectious gastroenteritis was prevalent, and we were able to conduct a study on the effect of preventing secondary infections of the infectious gastroenteritis using an aqueous solution of chlorine dioxide. As a result of examination of the four seasons since 2016, it was confirmed that there were no secondary infections of infectious gastroenteritis observed and there were no reported adverse events.

Finally, what are your future plans for this research?

We would like to examine our work's impact on infection prevention against the COVID-19 virus, which is wreaking havoc around the world at present. We are also interested in seeing the effect on the mutant strains that are now appearing on a regular basis ▶

Figure 1 Chlorine dioxide combats infections

New countermeasures against nosocomial infections

*A team of researchers based within the **Department of Pediatrics** at **Juntendo University** is investigating the efficacy and safety of chlorine dioxide against nosocomial infection to help develop improved infection countermeasures*

Nosocomial is a term used to describe any infection, disease or condition that originated in hospital. Ultimately, this means that a patient was admitted to hospital for one thing and is then afflicted by something else. It is an issue that people around the world have come to be increasingly aware of in recent years with the rise of more resistant bugs, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas aeruginosa* (MDRPA).

One example of a condition that can occur in patients who have been admitted to hospital is nosocomial pneumonia, which is a type of pneumonia that develops in patients around 48 hours after admission. Unfortunately, this form of pneumonia has a high mortality rate and occurs in a surprisingly large number of patients. For these reasons, researchers are intent on finding new and improved means of preventing nosocomial pneumonia from occurring, but also helping to develop treatments that help combat it in those incidents where it does occur.

One of the chief means of preventing nosocomial pneumonia (and the prevalence of MRSA and MDRPA) is through improving the sanitary conditions of hospitals, where the building undergoes extremely stringent disinfection and cleaning. However, in many ways, this is an uphill battle, as many infections are transmitted through droplets in the air (as we have seen with the recent COVID-19 pandemic), which are extremely difficult to combat, especially when the main preventive method is cleaning surfaces.

NOVEL AVENUES EXPLORED

It is with these challenges in mind that Professor Kaoru Obinata has embarked on a project employing a novel technique to combat nosocomial pneumonia and other conditions contracted in hospital. Based within the Department of Pediatrics at Juntendo University in Japan, Obinata leads a team that is exploring the safe and effective application of chlorine dioxide in medical settings. 'The novel element of the project comes from the fact that, in high concentrations, chlorine dioxide is toxic and can burn and/or severely irritate the skin and eyes of human beings, so it would not be an obvious avenue for exploration - especially in hospital patients, but from our perspective

Figure 2 New countermeasures against nosocomial infection

chlorine dioxide holds a lot of potential,' highlights Obinata.

The team of researchers have identified the potential success of chlorine dioxide as a countermeasure against nosocomial infections. 'In nosocomial infections, contact infections such as drug resistant bacteria and viruses become a problem. Furthermore, measures such as ventilation of air-conditioning and prevention of aerosol spread by shielding are being taken as measures against aerosol infection and air infection, which are also the problems we are seeing with the new COVID-19 virus,' observes Obinata. 'However, standard infection prevention measures alone leave viruses and bacteria in the environment. Secondary infections also occur in actual medical settings, making it difficult for clusters to occur.' He says they believe that it is possible to further reduce the risk of nosocomial infections by adding a more reliable infection countermeasure in spatial disinfection and virus removal using chlorine dioxide gas to the conventional infection countermeasures.

"It appears that chlorine dioxide aqueous solution can be applied in hospital settings for the prevention of a wide range of nosocomial infections"

IMPROVING EXISTING MEASURES

Of course, measures already exist within hospitals to prevent or counteract air and droplet infections. One of the chief methods is the use of high-efficiency particulate air (HEPA) filters, but even if the pathogenic microorganisms are captured by the filter, they are not sterilised or killed - meaning that the problems are not really solved in any meaningful way. There are also problems with the associated costs of HEPA filters, as well as the issues they cause regarding the need for secure spaces in hospitals - something difficult to achieve when HEPA filters are installed.

On the other hand, chlorine dioxide exists as a gas at room temperature and dissolves in water to form an aqueous solution. Chlorine dioxide gas and aqueous solution are known to have a sufficient effect on aerosol infection of the causative microorganism for nosocomial pneumonia and an improvement of its efficacy can be expected by using it in combination with the conventionally used HEPA filter. 'The infection route for nosocomial pneumonia is associated with aerosol infection when performing endotracheal suction operation in cases of respiratory management,' points out Obinata. He explains that as a preventive measure, the use of negative pressure and positive pressure chambers to prevent the diffusion and inflow of microorganisms are being carried out, in addition to the use of a closed suction system, the disinfection of hospital rooms, cleaning of the environment, ventilation and the use of HEPA filters. 'However, the installation of a negative pressure chamber is expensive and the HEPA filter cannot sterilise or kill the pathogenic organisms even if it can capture them. Alcohol is not effective for the disinfection of objects against norovirus and adenovirus while sodium hypochlorite has a problem in terms of producing carcinogens such as trihalomethane,' he outlines.

The low concentration of chlorine dioxide gas and aqueous solution are considered to be sufficiently effective against aerosol infection for the causative microorganism of nosocomial infection, and it is expected that when it is used in combination with a HEPA filter, it will be highly safe with good prevention effect and cost effectiveness.

FINDINGS TRANSLATED

So far, the team's investigations have shown that chlorine dioxide aqueous solution is effective against various bacteria, viruses and fungi at a lower concentration than sodium hypochlorite solution. However, it has also been found that low-concentration of chlorine dioxide gas is effective against airborne bacteria and viruses, as well as adherent bacteria and viruses. In mouse models, the team has shown that it is effective against aerosol infection for the influenza virus and against influenza-like illness in humans.

Chlorine dioxide aqueous solution has been proven to be effective against MRSA and MDRPA too - which seems to support its use against other nosocomial infections. 'Interestingly, the team's research has also found that it is effective against the new coronavirus (SARS-CoV-2) and the mechanisms of action that inhibits the binding of the spike protein of the SARS-CoV-2 on ACE2 through the action of chlorine dioxide has been verified,' confirms Obinata. 'Based on these results, it appears that chlorine dioxide aqueous solution can be applied in hospital settings for the prevention of a wide range of nosocomial infections.'

The next stage is for the researcher to find a means of ensuring that the concentration of chlorine dioxide can be kept to safe and constant levels so that the effects are beneficial and not harmful. Thus, they are working to clarify the installation location of the chlorine dioxide generating gel in hospital rooms, as well as the time at which the mechanism should be replaced. If these final pieces of the puzzle can be put together, there is a genuine chance that the findings can be translated into real-world settings, thereby saving the lives of patients around the world. ●

Project Insights

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The infection route for nosocomial pneumonia is associated with aerosol infection when performing endotracheal suction operation in cases of respiratory management, as a preventive measure the use of negative pressure and positive pressure chambers to prevent the diffusion and inflow of microorganisms are being carried out, in addition to the use of a closed suction system, the disinfection of hospital rooms, cleaning of the environment, ventilation and the use of HEPA filters. However, the installation of a negative pressure chamber is expensive, and the HEPA filter cannot sterilize or kill the pathogenic organisms even if it can capture them. Alcohol is not effective for the disinfection of objects against norovirus and adenovirus while sodium hypochlorite has a problem in terms of producing carcinogens such as trihalomethane.

The low concentration of chlorine dioxide gas and aqueous solution are sufficiently effective against aerosol infection for the causative microorganism of nosocomial infection, and it is expected that when it is used in combination with a HEPA filter, it will be highly safe with high prevention effect and cost effectiveness.

Chlorine dioxide aqueous solution has been proven to be effective against MRSA and MDRPA, which seems to support its use against other nosocomial infections. Based on these results, it appears that chlorine dioxide aqueous solution can be applied in hospital settings for the prevention of a wide range of nosocomial infections. The next stage is to find a means of ensuring that the concentration of chlorine dioxide can be kept to safe and constant levels so that the effects are beneficial and not harmful. Thus, we will work to clarify the installation location of the chlorine dioxide generating gel in hospital rooms, as well as the time at which the mechanism should be replaced[35] (Figure 2).

If these final pieces of the puzzle can be put together, there is a genuine chance that the findings can be translated into real-world settings, thereby saving the lives of patients around the world.

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Original Articles

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Application of the Bioabsorbable Polyglycolic Acid Sheet in Colorectal Anastomosis in Animal Models

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Objectives: Anastomotic complications after colorectal surgery are one of the most serious outcomes. To address this issue, this study used the newly developed bioabsorbable polyglycolic acid (PGA) sheet to assess its usefulness and safety using two approaches of double stapling technique (DST) after laparoscopic anterior resection (AR) in pig models.

Methods: Rectal intratissue pressure was assessed after DST anastomosis in two groups, i.e., with (PGA group) or without PGA sheet (nonPGA group), which was sandwiched between the anastomosis in the first approach. In the second approach, after laparoscopic DST anastomosis with PGA sheet attached at anvil side, the clinical short-term outcomes within 1 week and histological findings at 1 week after the surgery were evaluated.

Results: Assessment of rectal intratissue pressure showed a mean pressure of 9.28 kPa in the PGA group versus 5.78 kPa in the nonPGA group ($p = 0.39$). The results of clinical short-term outcomes revealed that there were no anastomotic complications. The results of histological findings in anastomotic bowel tissues with PGA sheet were not significantly different from those of the control case.

Conclusions: The bioabsorbable PGA sheet can be used for colorectal DST anastomosis in animal models and may be a valuable tool for this procedure.

Key words: colorectal anastomosis, bioabsorbable polyglycolic acid sheet, animal model, laparoscopic anterior resection, double stapling technique

Introduction

Anastomotic complications after colorectal surgery are the main cause of postoperative morbidity, mortality, impaired quality of life, and prolonged hospital stay, in addition to creating the risk for permanent stoma. Anastomosis complications generally include leakage, bleeding, and stenosis[1,2]. Anastomotic leakage (AL) is one of the major complications of colorectal surgery, especially in patients with rectal cancer. The occurrence of AL varies widely from 3% to 30% and increases the postoperative mortality rate[3–9]. An option that holds promise to control leakage is that of staple line reinforcement, although use of this reinforcement in general is not new, previously described for bariatric surgery[10,11], pancreatic surgery[12], and colorectal surgery[13,14].

Several previous studies have assessed bioabsorbable felt constituted from polyglycolic acid (PGA)/trimethylene carbonate. Franklin et al reported the first series of cases using staple line

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reinforcement in colon surgery, in which the bioabsorbable felt was loaded on a linear stapler[14]. The initial study results were very promising, with no bleeding and no significant leakage in a short-term follow-up. More importantly, no adverse outcomes have been observed in almost 3 years of continuous use. Consten et al reported early results with this approach that showed a decreased incidence of hemorrhage and leakage after gastric surgery[10]. Nguyen et al demonstrated that the use of bioabsorbable staple line reinforcement is safe and effective in the prevention of intraoperative staple line bleeding and postoperative gastrointestinal hemorrhage[11]. Several authors agree that the use of bioabsorbable felt as staple line reinforcement seems to be safe and may be useful in preventing AL, bleeding, and, potentially, intraluminal stenosis as well. The use of bioabsorbable staple line reinforcement may play an important role in high-risk patients undergoing colorectal surgery, which include those who use steroids, have a longer intraoperative time, or have cancer, immunosuppression, and contamination and other issues. However, Placer et al reported that bioabsorbable staple line reinforcement in colorectal surgery does not reduce the rate of pooled anastomotic complications[15].

A new, thinner (0.15 mm), reinforcement material constituted only from PGA (NEOVEIL; Gunze, Osaka, Japan) has been developed. This new type of sheet with only PGA allows a shorter absorption time, and the influence for tissue is less from this sheet. This study assessed the usefulness and safety of this new PGA sheet using the colorectal double stapling technique (DST) anastomosis during laparoscopic anterior resection (AR) via two approaches in animal models. Approach A involved the evaluation of usefulness, made by assessing the rectal intratissue pressure (RIP) after DST anastomosis. Approach B involved the evaluation of safety, made by assessing the clinical short-term outcomes of DST anastomosis with PGA sheet and evaluating histological findings

Materials and Methods

Ethics Statement

The miniature pigs were purchased from ZEN-NOH Nagano. The animal studies were approved by the Animal Review Board of Juntendo University (Approval number: 1323).

Surgical procedure

All procedures were performed under sterile conditions by surgeons responsible for the assigned procedure. The pigs were administered isoflurane via inhalation for anesthesia. A laparoscopic AR was performed with colorectal end-to-end anastomosis with DST. The resected colon was the approximately 5 cm. The anal side of rectum was cut using the Endo GIA Reinforced Reload with Tri-Staple Technology™ or Endo GIA Reload with Tri-Staple™ (Medtronic, Minneapolis, USA). The anvil was extracorporeally set into the oral side of the sigmoid colon. The colorectal anastomosis was intracorporeally performed using the EEA Circular Stapler with DST Series Technology™ (Medtronic, Minneapolis, USA).

Approach A

The animals, i.e., nine pigs were classified into two groups. The first was the PGA group. In this group, the anal side of rectum was cut using the Endo GIA Reinforced Reload with Tri-Staple Technology. The PGA sheet was used, which was sandwiched between the oral side of the colon and the anal side of the rectum in anastomosis (Figure 1). The second group was the nonPGA group. In this group, the anal side of rectum was cut using Endo GIA Reload with Tri-Staple. The PGA sheet was not used and was not sandwiched between the oral side of the colon and the anal side of the rectum in anastomosis. The measurement of RIP was performed using HANDY MANOMETER PG-100 102GP™ (Nidec Copal Electronics, Tokyo, Japan) in the rectum, intraluminally filling it to the point of just overflowing by injecting air after anastomosis and clamping the oral side of the colon (Figure 2).

Approach B

In this approach, laparoscopic AR was performed in four pigs. Rectal transection was performed using Endo GIA Reinforced Reload with Tri-Staple Technology in all pigs. In the first case, normal DST anastomosis was performed without using the PGA sheet. In the other three cases, the PGA sheet was extracorporeally attached to the anvil; subsequently, reinforced DST was performed (Figure 3(a)-(e)). After laparoscopic AR with DST, the clinical courses were observed for 1 week. Furthermore, second-look surgery was performed to observe the

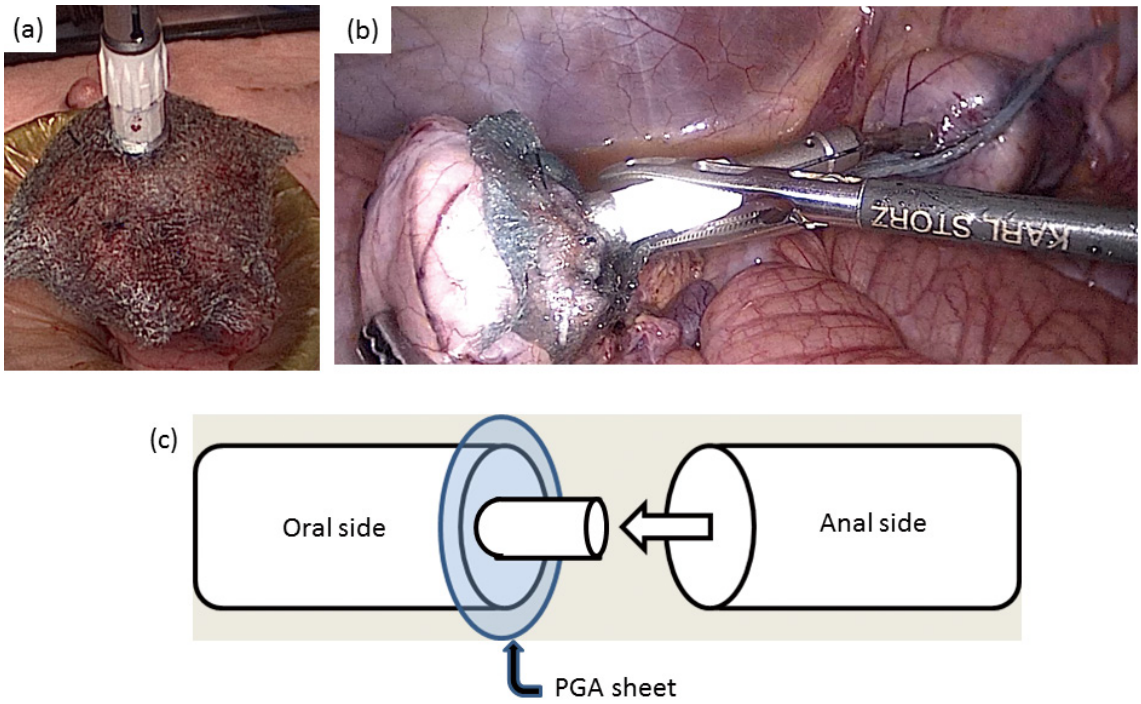

Figure 1 Colorectal anastomosis in the PGA group (Approach A)
(a) The oral side of colon is covered with the PGA sheet outside of the abdominal space. (b) The PGA sheet is sandwiched between the oral side of the colon and the anal side of the rectum in anastomosis. (c) The schema of anastomosis in the PGA group.

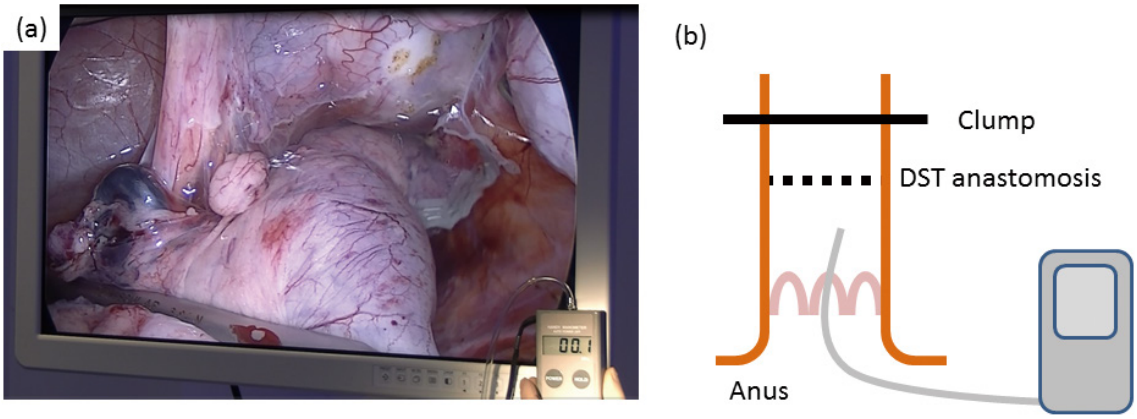

Figure 2 The schema of the measurement of RIP
(a) The measurement of RIP was performed using HANDY MANOMETER PG-100 102GP. (b) The schema of the measurement of RIP. A sensor in the rectum was used for pressure measurement. The colon was clumped on the oral side of the anastomosis.

intracorporeal findings 1 week after the first operation, and anastomoses were resected and assessed histologically with hematoxylin and eosin stain. Anastomotic factors were clinically and histologically assessed.

Statistical analysis

Comparison between the RIP of the PGA group and the nonPGA group was performed using Mann–Whitney U test. This analysis was performed using the JMP software (version 12.0, SAS Institute). All calculated p values were two-sided and $p < 0.05$ was considered statistically significant.

Results

Approach A: Measurement of RIP

There were five pigs in the PGA group and four in the nonPGA group. The mean RIP was 9.28 kPa

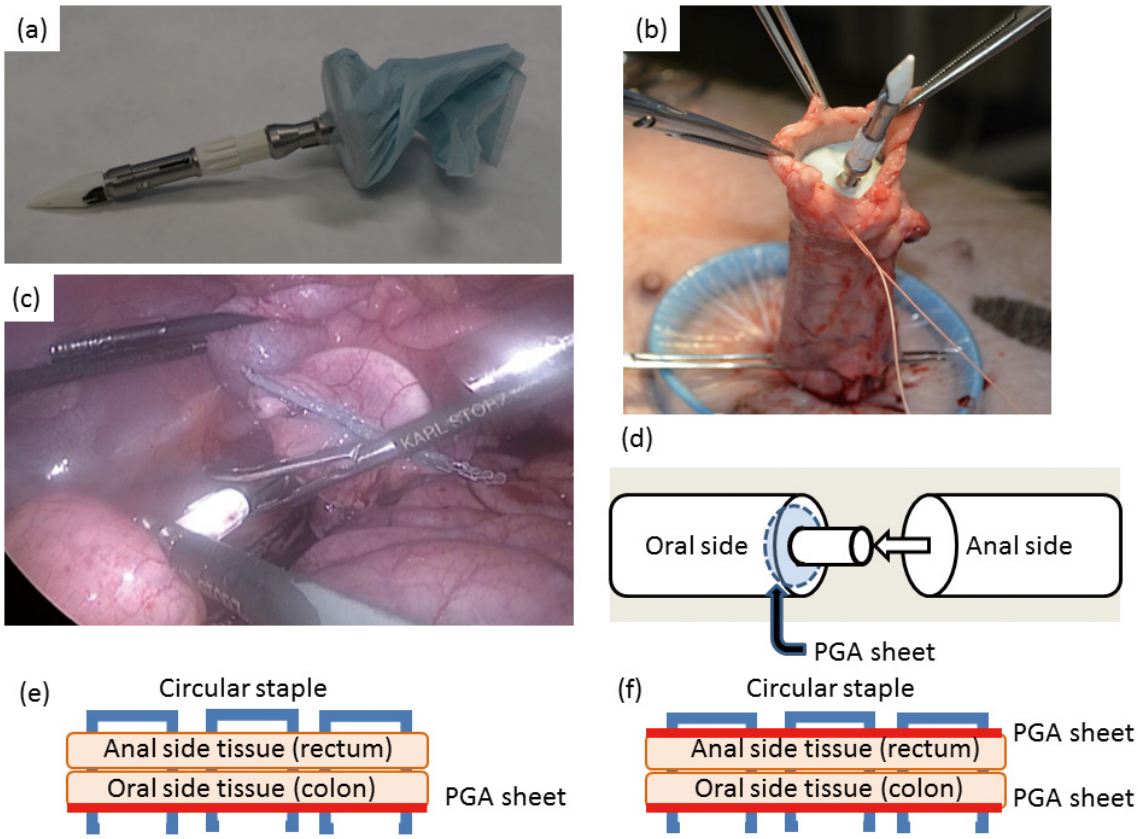

Figure 3 Colorectal anastomosis in the PGA group (Approach B)
(a) The anvil was covered with the PGA sheet. (b) This anvil was set into the oral side of colon. (c) The PGA sheet was on the oral side of the colon with the anvil. (d) The schema of anastomosis. (e) The schema of staple and PGA sheet in this approach. One PGA sheet was used on the oral side. (f) The schema of staple and PGA sheet to be used in the future. Two PGA sheets were used on the oral and the anal side.

for the PGA group versus 5.78 kPa for the nonPGA group, which did not differ statistically (p = 0.39) (Figure 4).

Approach B: Clinical Short-Term Outcomes

All four pigs were alive and had no event of clinical findings, such as fever, infection of incisional wound, or appetite loss, after undergoing laparoscopic AL with DST anastomosis. Although little postoperative adhesion was expected around the anastomosis during the second-look surgery, no anastomotic complications were evident in the abdominal cavity, such as bleeding, stricture formation, or leakage (Table 1).

Approach B: Histological Findings (Table 1)

There was no histological leakage in the anastomosis. Histological findings showed increased fibroblasts and neutrophils in both the PGA and the control groups. Slightly more granulation formation was noted around the anastomosis in the PGA cases than in the control case. Also, in the PGA group, the PGA sheet was observed to have remained in the anastomosis. These two groups showed no other significant differences except that of the remaining the PGA sheet fibers (Figure 5). In addition, abscess formation was found around the anastomosis in one of the PGA cases. However, there was some distance between the abscess formation and PGA sheet. Moreover, no necrotic tissue was observed near the anastomosis.

Discussion

Recently, several studies have reported the effectiveness of staple line reinforcing material for the prevention of anastomosis complications. This study assessed the usefulness and safety of a newly available PGA sheet by two approaches in animal models to show the potential of this new PGA sheet for the DST anastomosis of the colorectal surgery. Approach A was aimed to assess its utility for the reinforcement of the circular anastomosis. Approach

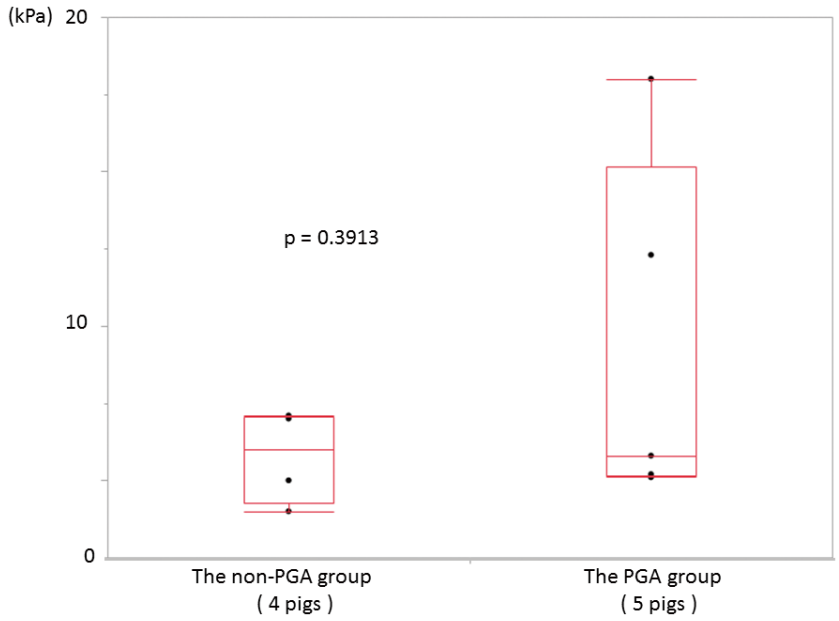

Figure 4 Measurement of RIP
The mean RIP was 9.28 kPa for the PGA group versus 5.78 kPa for the nonPGA group, which did not reach statistical significance (p = 0.3913).

Table 1 Result of Approach B

	PGA	Clinical complications of anastomosis			Histological findings in anastomotic tissue		
		leakage	bleeding	structure	leakage	Necrotic tissue	Abscess formation
Control case	(−)	(−)	(−)	(−)	(−)	(−)	(−)
Case 1	(+)	(−)	(−)	(−)	(−)	(−)	(+)
Case 2	(+)	(−)	(−)	(−)	(−)	(−)	(−)
Case 3	(+)	(−)	(−)	(−)	(−)	(−)	(−)

B was aimed at addressing its safety for use in colorectal anastomosis.

In approach A, the aim was to examine the utility by measurement of RIP. Results showed not statistical differences, although the mean RIP of the PGA group was higher than that of the nonPGA group. This finding showed that the PGA sheet might reduce the AL in colorectal surgery. Naito et al reported that the high rates of complete staple formation were important for reducing anastomosis failure[16]. They reported that reinforcement with the PGA sheet results in higher rates of complete staple formation in the approach of linear-staple on linear-staple site. In this animal model, there might be the same reinforcement with the PGA sheet in circular-staple on linear-staple site.

In approach B, laparoscopic AR was performed to clinically and histologically assess the safety of short-term outcomes of DST anastomosis postoperatively. This evaluation showed that the PGA sheet may be safe for use in colorectal surgery. Of the three pigs examined, none was found to have complications from the anastomosis. Histologic findings showed the PGA sheet remained in the anastomosis and that granulation formation had occurred around the anastomosis because of using the PGA sheet because the sheet provided scaffolds for early tissue repair. Well-built barriers of inflammatory granulation developed around the site of the anastomosis. Takagi et al showed this scaffold reduced the postoperative rate of pancreatic fistula[12]. Early enclosure of the pancreatic leak by the granulation formation may have been achieved, and the barrier formed by the abundant fibroblast infiltration in the scaffold of the PGA sheet likely prevented the postoperative pancreatic fistula.

This study has several limitations. First, the sample size was small and thus could not demon-

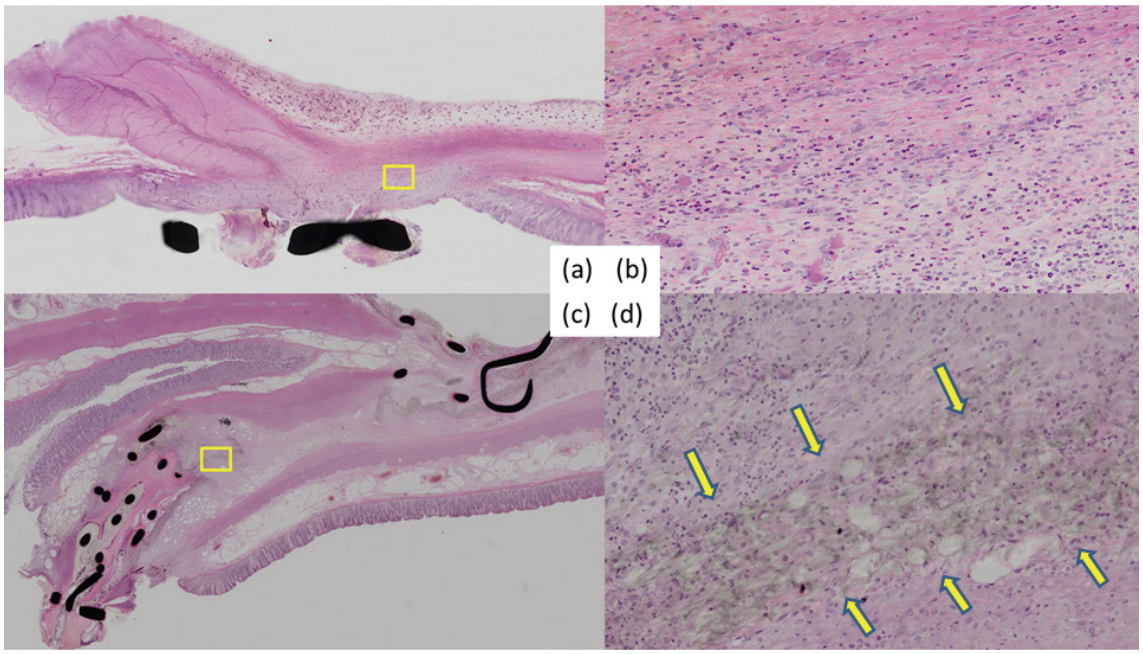

Figure 5 Histological findings of anastomosis (Approach B)
(a) Histological findings (hematoxylin and eosin stain). (b) Anastomosis of the control case. (c) Anastomosis with the PGA sheet, with obvious remaining PGA material. (d) The PGA sheet is represented as the space between the arrows.

strate statistical difference. The results of our approaches were the higher credibility because we had used the big animal like pigs. Animal studies with a larger sample size are too difficult to continue ethically. Our results suggested that this approach was risk-free. In the future, we plan to conduct a study that confirms the PGA sheet's effectiveness in humans. The RIP was dependent on the resting pressure of the anus; hence, the resting pressure of the anus should be measured to obtain more accurate data. Multiple RIP measurements should be made for reproducibility. However, in our study, we measured the RIP only at the point when the injected air had just overflowed. The anastomosis was broken only during one measurement, and we had only one chance of measurement per anastomosis. The duration of follow-up was short (1 week), whereas ALs may be detected anywhere from 3 to 45 days postoperatively[17, 18]. In addition, there appears to be two peaks of when the diagnosis is made of anastomotic leaks. When leakages observed clinically, the median postoperative time of diagnosis is 7 days; when leaks are diagnosed radiographically, the median postoperative time is 16 days[8]. In this approach, anastomotic leaks were assessed by only clinical findings. None of the pigs had clinical complications associated with anastomosis failure; therefore, the authors posited that short-term outcomes of AR might be acceptable. The other key histological finding was the abscess formation in one pig in approach B. In approach B, the PGA sheet was set intrarectally for attaching it to the mucosal surface, not within the tissue. Although there was no PGA sheet in the abscess, this finding showed that the PGA was able to lead to surgical site infection. Few studies have reported that the PGA sheet was attached to the mucosal surface[19]. However, they did not report the histological findings. To our knowledge, our report is rare in that the PGA sheet in our study was attached to the mucosal surface of the anastomotic site of the circular stapler and assessed on the basis of histological findings. The relationship between with the clinical findings and abscess formation was unclear and must be verified. Finally, the locations of the PGA sheet in these two approaches were different. In approach A, the method to use the PGA sheet was the simplest, in which it was sandwiched between the oral and the anal side. In approach B, we considered using two PGA sheets, similar to the more clinical Endo GIA Reinforced Reload with Tri-Staple Technology (Figure 3(f)).

However, our adaptation of circular Stapler with PGA sheet made it easy to slip blindly from the anus to the stump through the rectum and increased the risk of obstruction and stenosis. More PGA sheets allowed for better reinforcement of anastomosis by providing more scaffolds for early tissue repair. However, an excessive number of PGA sheets could increase the risk of obstruction and stenosis. Therefore, we used one PGA sheet, as shown in Figure 3(e). We planned to create a new device model that uses two PGA sheets for both the circular stapler and anvil. If we use a PGA sheet for the circular stapler, the PGA sheet might shift during circular stapler insertion in the anus. We thought that the PGA sheet processing was required to solve this problem. Therefore, we used the PGA sheet only in the anvil in approach B. In the future, two PGA sheets can be used more safely if a new device that employs the combination of a circular stapler and PGA sheet is developed.

In conclusion, this study experimentally assessed the application of a bioabsorbable PGA sheet in colorectal DST anastomosis clinically and histologically. Use of this sheet facilitated the setting to anvils because it was added outside the abdominal space. This study also showed the possibility that anastomosis failure may be reduced in human colorectal surgery. It is the belief of these authors that this safe device may be a valuable tool for colorectal anastomosis.

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Author contributions

Study concept and design, S Kawano, K Sakamoto; data acquisition, Y Tsuchiya, R Tsukamoto, Y Kojima; Data analysis and interpretation: S Motegi, K Kure K Sugimoto; Drafting of manuscript: S Kawano; Critical revision: M Takahashi, Atsushi Okuzawa, Kazuhiro Sakamoto; All authors read and approved the final manuscript.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

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Original Articles

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Validity of Diaphragm Volume Measurements Using Three-Dimensional Computed Tomography

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Objectives: The aim of this study was to measure the diaphragm volume using three-dimensional computed tomography (3D-CT) and verify its validity.

Design: This was a retrospective study of existing samples.

Methods: Participants comprised five male patients, aged 65–70 years, who underwent preoperative chest CT (with a slice thickness of 0.5 mm) before coronary artery bypass surgery. The diaphragm was selectively extracted using a workstation to reconstruct a stereoscopic image, and the total muscle volume was measured. To confirm the accuracy and reproducibility of diaphragm muscle volume measurements on CT, all cases were measured three times by two observers, and intraclass correlation coefficients (ICCs) and interobserver correlations were determined.

Results: Observers #1 and #2 reported an average diaphragm volume of 256.7±33 cm^3 and 259.3±36 cm^3, respectively. The ICC analyses yielded Cronbach's alphas of 0.992 and 0.981 from both observers, and the interobserver correlation was 0.991. The ICC of a single measurement and the average measurement was 0.984 (95% confidence interval: 0.998–0.884) and 0.992 (95% confidence interval: 0.999–0.939), respectively.

Conclusions: To our knowledge, this study is the first to standardize the method for measuring the total diaphragm volume and examine the reproducibility and validity of the new method. The diaphragm could be selectively extracted and reconstructed. Measurement of the total diaphragm muscle volume using a workstation to reconstruct a stereoscopic image is feasible and highly reproducible. This technique can be reliably employed to evaluate diaphragm volume, thickness, and morphology.

Key words: diaphragm, diaphragm volume, respiratory muscles, three-dimensional computed tomography, workstation

Introduction

The diaphragm is the main muscle involved in respiration, and functions during breathing[1]. Understanding its anatomy is important for surgical treatment. Furthermore, an accurate evaluation of respiratory function during surgery performed under general anesthesia is an absolute condition for safely performing intraoperative and postoperative management and directly affects the surgical outcome, especially in thoracic surgery with thoracotomy[2,3]. Mechanical ventilation (MV) is necessary during surgery, as well as after surgery in the intensive care unit (ICU)[4–6]. Multiple studies have suggested that MV has a significant effect on the respiratory system, especially the respiratory muscles[7,8]. In animal experiments, inactivity of the diaphragm on MV and passive ventilation lasting for more than 18 hours induced atrophy in the muscle fibers of the diaphragm[7,9]. This is also the case for the human diaphragm; MV for 18–69 hours resulted in significant atrophy of the human diaphragm muscle fibers[10]. A clinical study that measured muscle thickness using echography

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found that the overall rate of decrease in the thickness of the patient's diaphragm averaged 6% per day of MV[11]. Furthermore, in clinical practice, diaphragm thickness has been shown to decrease rapidly within a few days of MV in approximately 40% of patients[10, 12]. Recently, diaphragm thickness measured during resting tidal breathing, in a cycle of spontaneous breathing, was shown to predict extubation success[13, 14]. However, the rate of reintubation within 24–72 hours of planned extubation ranged from 2% to 25%, with medical, pediatric, and multidisciplinary ICU patients at the highest risk[15, 16].

To date, diaphragm volume evaluations, which are also commonly used in the context of research areas such as sarcopenia, physical development, and aging[17, 18], have only been determined using muscle thickness on echography. However, using echography alone to measure muscle thickness is inadequate[19]; echography cannot be used to evaluate the overall morphology and volume of the diaphragm. By contrast, three-dimensional computed tomography (3D-CT) can clearly reveal the cross-sectional thickness and can also be used to evaluate morphology and volume. Sometimes, it is very difficult to differentiate the diaphragm from the liver and that is why some researchers report only the left diaphragm volume associated with the pulmonary function test result[20]. To our knowledge, several studies used workstation software applications to evaluate diaphragm volume[20–22]; however, until now, there has been no definitive standard for diaphragm volume measurements[23]. Therefore, this study aimed to standardize the method of measuring diaphragm volume using 3D-CT with new criteria and to examine the validity of the new method.

Materials and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study design was approved by the institutional review board of Juntendo University School of Medicine (approval number: 20-342). For retrospective medical record surveys that handle only existing samples, instead of omitting informed consent, information on the implementation of research, including the purpose of the research, is posted by the Department of Cardiovascular Surgery on the Juntendo University website. We guaranteed the opportunity for the research subjects to refuse. The information disclosure document used was approved by the institutional review board of Juntendo University School of Medicine. Information that can identify the research subjects is not included. In addition, we did not use the samples of the research subjects for purposes other than research purposes.

Research subjects

This was a retrospective study of existing samples. The participants comprised patients who were hospitalized or admitted to the Department of Cardiovascular Surgery at Juntendo University School of Medicine between June 2017 and January 2019. The selection criteria were as follows: patients underwent chest CT to evaluate the graft before coronary artery bypass grafting during hospitalization, and CT was performed with a 0.5-mm slice thickness (320 row multidetector Aquilion ONE ViSION Edition, Canon Medical Systems, Japan). Five patients (all male: age, 68.2±1.5 years; weight, 70.7±8.1 kg; height, 164.7±3.1 cm; BMI, 25.96±2.16 kg/m^2; BSA, 1.77±0.10 m^2) met the abovementioned selection criteria during the study period.

As 3D-CT can evaluate, not only the difference in thickness but also the morphology and total volume, this method was used in the current study (Figure 1).

3D-CT assessment of the diaphragm

Diaphragm volume was measured based on the method used by previous researchers[24, 25]. To evaluate the total volume of the diaphragm as much as possible, in this study, we standardized the method of measuring diaphragm volume using 3D-CT with a new standard and verified the validity of the new method. To confirm the accuracy and reproducibility of diaphragm muscle volume measurements on CT, measurements were recorded three times by two observers, and intraclass correlation coefficients (ICCs) as well as interobserver correlations were determined. The detailed explanation of this method is provided below.

All CT data were imported into the workstation software application, Attractive Medical Image Processor (PixSpace Co. Ltd., Japan) (Figure 2a).

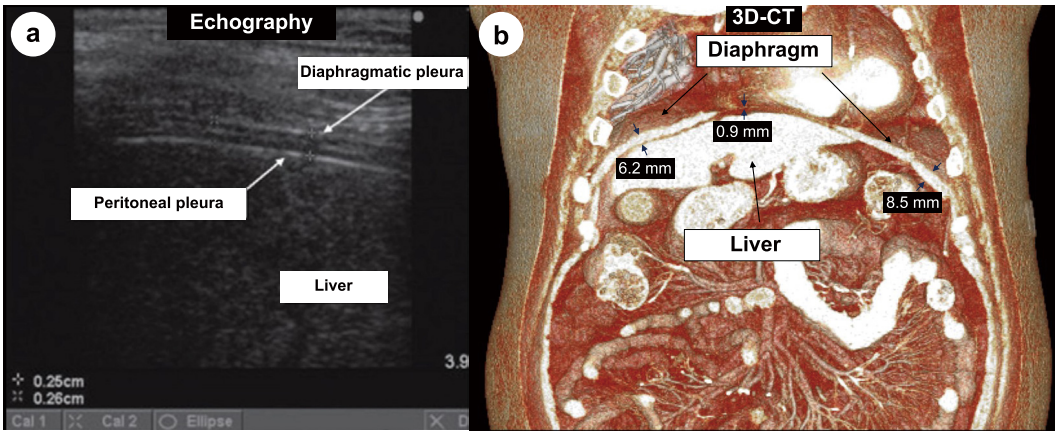

Figure 1 Echography and three-dimensional computed tomography (3D-CT). (a) The diaphragm, peritoneum, and liver[11] on echography. (b) The diaphragm and liver on 3D-CT. The diaphragm was selectively extracted from the CT data using a workstation to reconstruct a stereoscopic image, and the total muscle volume was measured. To confirm the accuracy and reproducibility of the diaphragm muscle volume measurements on CT, all cases were measured three times by two observers, and the intraclass correlation coefficients for each observer and interobserver correlations were determined.

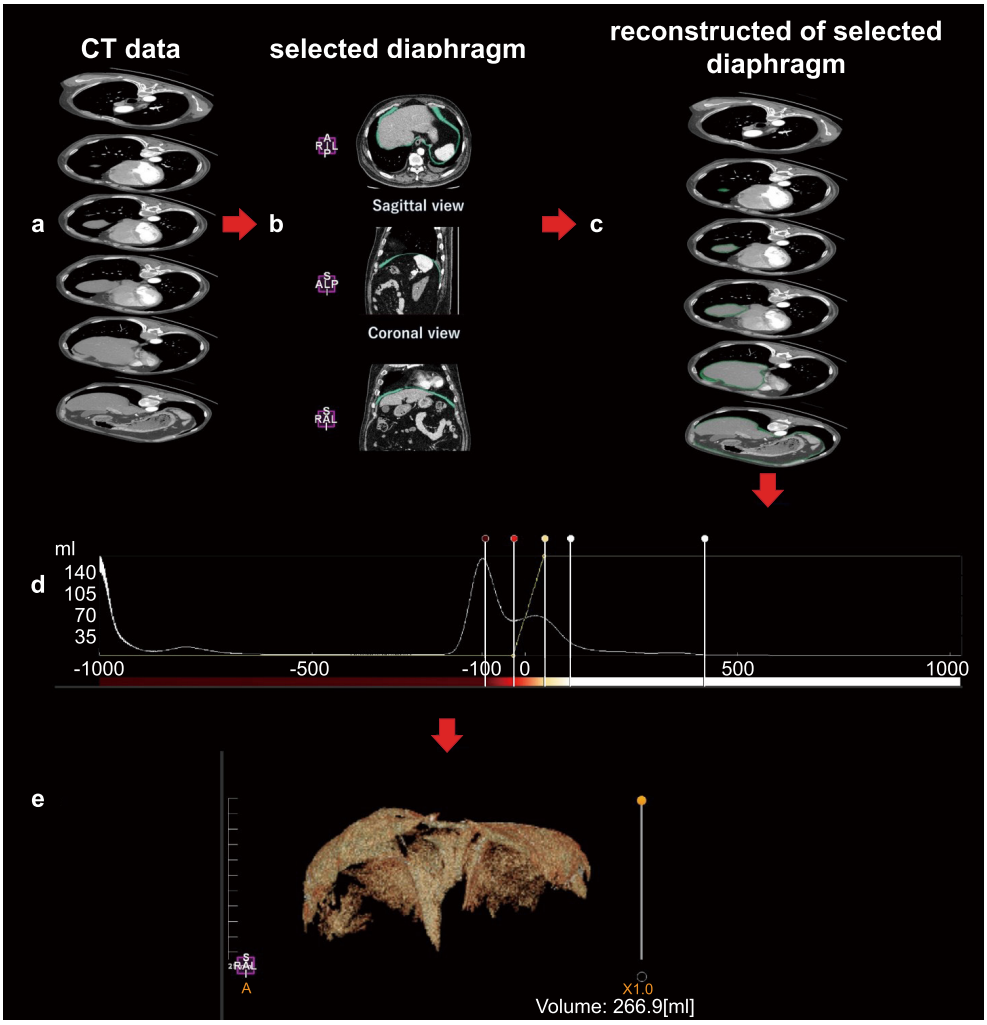

Figure 2 Operating steps for the assessment of the diaphragm volume during deep inspiration. (a) All computed tomography (CT) data are imported into the workstation software. (b) The diaphragm is selected and extracted. (c) The diaphragm is reconstructed. (d) The color mapping operation screen is shown. (e) The volume is measured.

For each slice, the diaphragm was selected and extracted (Figure 2b). The diaphragm was then reconstructed using the contour trace function (Figure 2c). We performed color mapping to ensure that the entire diaphragm was visible (Figure 2-d), and the volume was measured using the 3D volumetry function (Figure 2e). Each of these steps is detailed below.

For diaphragm selection, it was easy to select the diaphragm when there were no surrounding organs (Figure 3d). However, it was difficult to identify the diaphragm when surrounding organs were present, especially at locations in which the diaphragm and liver were attached (Figure 4). The following rules were used to standardize the selective extraction of the diaphragm: (1) The selection was first based on the coronal view and confirmed in the axial and sagittal views after extraction (Figure 3); (2) locations in which the diaphragm was attached to the chest wall and heart were not selected (Figure 3a and 3c); and (3) the zoom function was used more carefully to

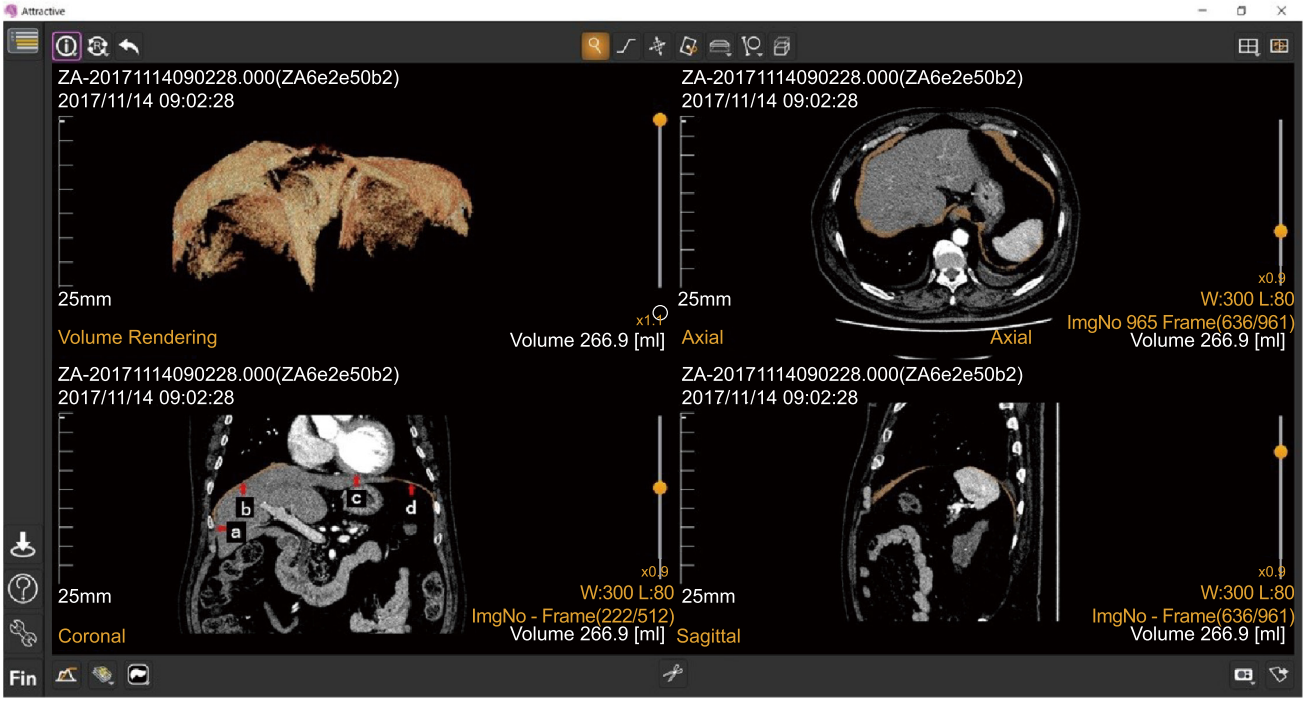

Figure 3 Diaphragm selection operation screen showing coronal, axial, and sagittal views of the diaphragm. (a) The location of the diaphragm attached to the chest wall. (b) Location of the diaphragm attached to the liver. (c) Location of the diaphragm attached to the heart. (d) Location of the diaphragm without attachment to any surrounding organs.

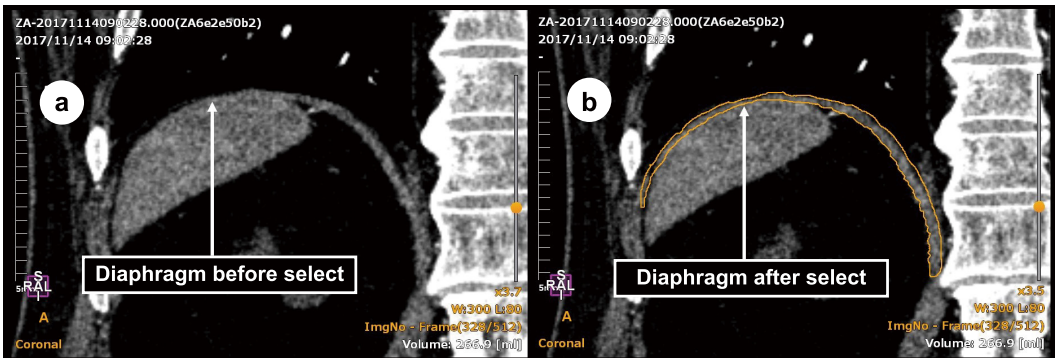

Figure 4 Diaphragm selection operation screen showing a coronal view of the diaphragm (a) before selection and (b) after selection. Diaphragm extraction: The diaphragm was separated from the heart, lungs, liver, and other surrounding tissues and then reconstructed (Figure 3 and Figure 4). Color mapping: Color mapping was performed such that the entire diaphragm was visible (Figure 2b).

guide selection at the locations in which the diaphragm was attached to the liver and chest wall (Figure 3b and 4b).

For the removal of invisible areas, the 3D cutting function was used to remove air and fat tissue, which were selectively extracted from the diaphragm in invisible areas.

For the diaphragm volume measurement, in the final step, the diaphragm volume was measured using the 3D volumetry function (Figure 3), and observer #1 recorded three separate measurements for each case at AZE Virtual Place (AZE Co., Tokyo, Japan) using the same method. ICCs and interobserver correlations were then determined.

Statistical analyses

SPSS software (version 25.0; SPSS Statistics, IBM Corp., Armonk, NY, USA) was used to perform the statistical analyses. All data are expressed as the mean ± standard deviation (SD) or 95% confidence intervals (CIs), and the significance level was set at less than 1%. The ICCs of each observer and between both observers were analyzed. The one-way random effects model with participant effects as random was applied to the ICCs of each observer, and a two-way random effects model with both participant effects and measure effects as random was applied to the ICCs of the interobserver differences. ICCs were interpreted following guidelines by Koo and Li (2016)[26] and classified as follows: below 0.50, poor; between 0.50 and 0.74, moderate; between 0.75 and 0.90, good; and above 0.90, excellent.

Results

Age, weight, height, body mass index, and body surface area were normally distributed. In all cases, the diaphragm could be separated from the heart, lungs, liver, stomach, and other surrounding tissues, and the diaphragm volume could be evaluated (Figure 5 and 6).

Table 1 summarizes the detailed results and the average of all measurements by workstation software PixSpace according to the observer. The intraobserver agreement for the three measurements was excellent for observer #1, based on a Cron-

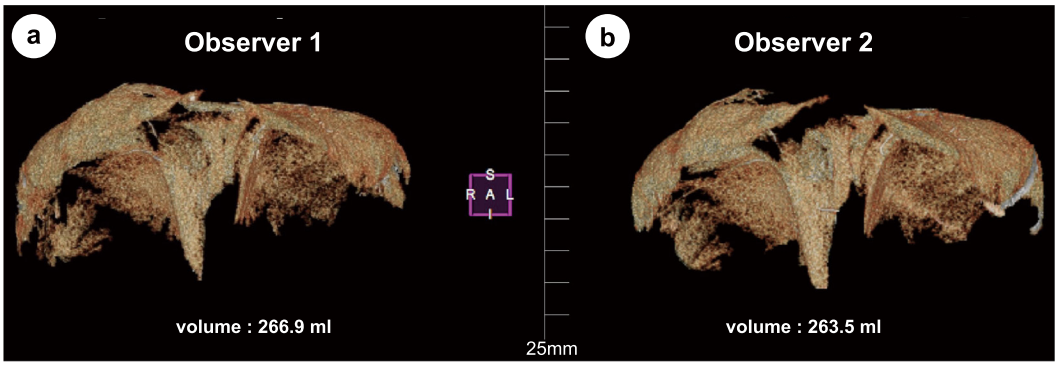

Figure 5 Diaphragm morphology and volume after reconstruction in case #4. The first measurement of (a) observer #1 and (b) observer #2 by workstation software PixSpace.

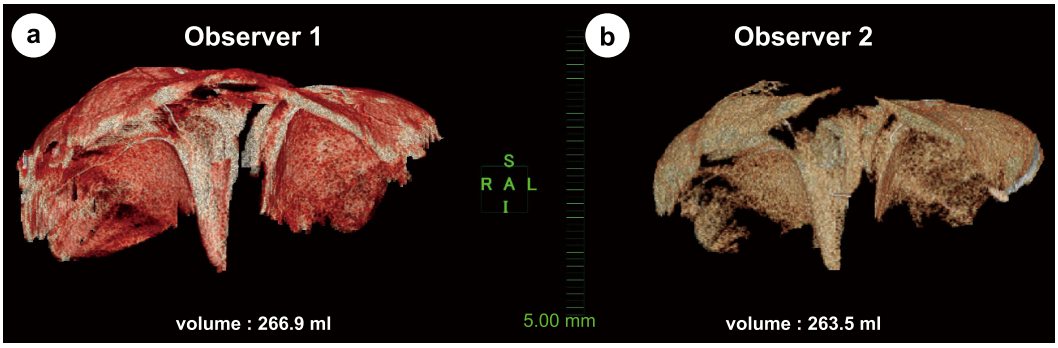

Figure 6 Diaphragm morphology and volume after reconstruction in case #4. The first measurements of (a) observer #1 by workstation software AZE and (b) observer #2 by workstation software PixSpace.

bach's alpha of 0.992, single-measurement ICC of 0.974 (95% CI: 0.997-0.892), and average-measurement ICC of 0.991 (95% CI: 0.999-0.961) (Table 2). The intraobserver agreement for the three measurements was also excellent in observer #2, based on a Cronbach's alpha of 0.981, single-measurement ICC of 0.955 (95% CI: 0.995-0.817), and average-measurement ICC of 0.984 (95% CI: 0.998-0.931) (Table 3). The interrater agreement, summarized in Table 4, showed a strong correlation among the measurements recorded by the two observers, with a Cronbach's alpha of 0.991, single-measurement ICC of 0.984 (95% CI: 0.998-0.884), and average-measurement ICC of 0.992 (95% CI: 0.999-0.939).

Table 5 summarizes the detailed results and the average of all measurements by workstation software AZE to Observer #1. The intraobserver agreement for the three measurements by AZE

Table 1 Detailed results and average diaphragm volume across all measurements (first, second, and third) for observer #1 and observer #2 according to workstation software PixSpace

n=5	Observer #1				Observer #2			
	First (cm^3)	Second (cm^3)	Third (cm^3)	Mean (cm^3)	First (cm^3)	Second (cm^3)	Third (cm^3)	Mean (cm^3)
1	309.7	302.1	307.2	306.3	314.2	307.5	318.0	313.2
2	235.3	240.3	244.3	240.0	258.6	239.8	249.3	249.2
3	236.5	230.0	242.2	236.2	233.9	245.5	242.5	240.6
4	266.9	279.2	277.5	274.5	263.5	285.7	272.3	273.8
5	231.5	221.3	226.2	226.3	224.8	215.8	218.6	219.7
Mean	256.0	254.6	259.5	256.7	259.0	258.9	260.1	259.3
SD	33	35	33	33	35	37	38	36

Abbreviation: SD, standard deviation.

Table 2 Intraclass correlation coefficients for observer #1 by workstation software PixSpace

Measurement	ICC	95% CI		F-test with true value=0				Reliability statistics	
		Lower	Upper	Value	df1	df2	*P*-value	Cronbach's alpha	n
Single	0.974	0.892	0.997	115.413	4	10	<0.001	0.992	3
Average	0.991	0.961	0.999	115.413	4	10	<0.001		

A one-way random effects model was applied with participant effects as random.
Abbreviations: CI, confidence interval; df, degrees of freedom; ICC, intraclass correlation coefficient.

Table 3 Intraclass correlation coefficients for observer #2 by workstation software PixSpace

Measurement	ICC	95% CI		F-test with true value=0				Reliability statistics	
		Lower	Upper	Value	df1	df2	*P*-value	Cronbach's alpha	n
Single	0.955	0.817	0.995	64.392	4	10	<0.001	0.981	3
Average	0.984	0.931	0.998	64.392	4	10	<0.001		

A one-way random effects model was applied with participant effects as random.
Abbreviations: CI, confidence interval; df, degrees of freedom; ICC, intraclass correlation coefficient.

Table 4 Interrater agreement between measurements recorded by the two observers with workstation software PixSpace

Measurement	ICC[a]	95% CI		F-test with true value=0				Reliability statistics	
		Lower	Upper	Value	df1	df2	*P*-value	Cronbach's alpha	n
Single	0.984[b]	0.884	0.998	117.593	4	4	<0.001	0.991	2
Average	0.992	0.939	0.999	117.593	4	4	<0.001		

[a] Type A ICCs using an absolute agreement definition.
[b] The estimator was the same, regardless of whether the interaction effect was present.
A two-way random effects model was applied with both participant effects and measure effects as random.
Abbreviations: CI, confidence interval; df, degrees of freedom ICC, intraclass correlation coefficient.

Table 5 Detailed results and average diaphragm volume across all measurements (first, second, and third) for observer #1 by workstation software AZE

n=5	Observer #1(AZE)			
	First (cm^3)	Second (cm^3)	Third (cm^3)	Mean (cm^3)
1	309.6	308.4	301.7	306.6
2	238.3	235.3	238.9	237.5
3	227.7	222.6	229.5	226.6
4	258.6	272.8	260.0	263.8
5	230.5	224.2	227.7	227.5
Mean	252.9	252.7	251.6	252.4
SD	34	37	31	34

Abbreviation: SD, standard deviation.

Table 6 Intraclass correlation coefficients for observer #1 by workstation software AZE

Measurement	ICC	95% CI		F-test with true value=0				Reliability statistics	
		Lower	Upper	Value	df1	df2	*P*-value	Cronbach's alpha	n
Single	0.982	0.922	0.998	162.011	4	10	<0.001	0.992	3
Average	0.994	0.972	0.999	162.011	4	10	<0.001		

A one-way random effects model was applied with participant effects as random.
Abbreviations: CI, confidence interval; df, degrees of freedom; ICC, intraclass correlation coefficient.

Table 7 Interrater agreement between measurements recorded by the two observers between different workstations

Measurement	ICC[a]	95% CI		F-test with true value=0				Reliability statistics	
		Lower	Upper	Value	df1	df2	*P*-value	Cronbach's alpha	n
Single	0.956[b]	0.635	0.995	63.902	4	4	<0.001	0.984	2
Average	0.978	0.776	0.998	63.902	4	4	<0.001		

[a] Type A ICCs using an absolute agreement definition.
[b] The estimator was the same, regardless of whether the interaction effect was present.
A two-way random effects model was applied with both participant effects and measure effects as random.
Abbreviations: CI, confidence interval; df, degrees of freedom ICC, intraclass correlation coefficient.

was excellent for Observer #1, based on a Cronbach's alpha of 0.992, single-measurement ICC of 0.982 (95% CI: 0.998-0.922), and average-measurement ICC of 0.994 (95% CI: 0.999-0.972) (Table 6). The interrater agreement, summarized in Table 7, showed a high correlation between the measurements taken by the two observers between different workstation software, with a Cronbach's alpha of 0.984, single-measurement ICC of 0.956 (95% CI: 0.995-0.635), and average-measurement ICC of 0.978 (95% CI: 0.998-0.776).

Discussion

We performed an accurate evaluation of diaphragm morphology and volume using 3D-CT. Although, to our knowledge, this is not the first study to measure the total volume of the diaphragm[20-22], this study was the first to standardize the method for measuring the total diaphragm volume and examine the reproducibility and validity of the new method. Using 3D-CT, we were able to confirm the morphological evaluation of the entire diaphragm, including its form, thinness, and volume.

The diaphragm muscle volume reported in a previous study[21] was smaller than the mean diaphragm volume measured by our method. Although this could be due to differences in patient medical history and characteristics, as well as the software used, the difference in measurement method is also likely to be a major reason. Image analysis software generally use measurement methods that involve semi-automatic tracking and selection. This increases measurement speed, but the anatomy of the diaphragm makes it is difficult

to perform complete selective extraction, due to the requirement for manual selection and data extraction. Thus, accurate measurement depends on the selection criteria as well as the operating ability of the measurer. Moreover, 1-mm slices were used in previous studies[21], whereas 0.5-mm slices were used in this study. We believe that manual selection and use of 0.5-mm slices enabled better evaluations; furthermore, in contrast to the previous study[21], we have described the operation procedure and selection criteria in detail to make measurement easier to reproduce. Fluctuations in intraclass and interrater correlations are likely to be due to differences in manually selected areas. We believe that this difference will be smaller if the technical training and operating procedures are standardized.

Although it remains uncertain whether the volume measured using the workstation Attractive Medical Image Processor software application, reflects the true volume of the diaphragm, other researchers have already confirmed that the volume of some organs measured on CT was the same as the actual volume. For example, in 1979, Heymsfield et al[27]. were the first to accurately measure liver, kidney, and spleen volumes and masses using computerized axial tomography. The authors showed that this method was accurate by comparing the estimated and actual weights obtained at autopsy. The determination of liver volume on CT is now widely used in the field of liver surgery[28] and transplantation[29]. In 2002, Surusuk et al[30]. suggested that Heymsfield's method[27] is also reliable for the volumetric analysis of liver segments. Therefore, the diaphragm volume assessment in this study could have clinical value. However, this study was limited by its small sample size and retrospective nature. Consequently, the results were not sufficient to show the clinical usefulness of 3D-CT assessment of the diaphragm volume in comparison to that with a current reference imaging method, such as sonography.

In previous studies, conventional fluoroscopy, ultrasound, and magnetic resonance imaging (MRI) have been used to evaluate diaphragmatic functionality[31, 32]. B- and M-mode ultrasound can easily diagnose diaphragmatic dysfunction and compare changes during the follow-up period[33], as well as provide real-time evaluation. It is often the imaging modality of choice and is a suitable bedside procedure. However, ultrasound is limited by operator dependency[33, 34] and cannot be used to evaluate the total diaphragm volume.

Several studies have shown that it is possible to evaluate diaphragm morphology and function using MRI[35, 36]. MRI is a radiation-free technique that can provide static or dynamic evaluations, with the benefit of a wider field of view and more detailed soft tissue characterization[37]. However, MRI, like ultrasound, cannot be used to evaluate the total diaphragm volume. Furthermore, the wide use of MRI is restricted by its limited availability, difficult scheduling, difficult patient preparation, and high costs[38]. Because of its wider availability, CT scans are more commonly used as frontline imaging tests than MRI scans[36].

The present method also allows for the full use of existing CT data. The CT data used in this study were not initially collected to evaluate the diaphragm; rather, the CT data were obtained preoperatively. As existing CT data can be used, the problem of radiation exposure is reduced. As the conditions of the study subjects were limited, the postoperative diaphragm volume could not be measured in this study; however, changes in the diaphragm volume can be examined using existing CT data before and after surgery. Diaphragm volume measurements on 3D-CT can be expected to best combine the benefits of MRI and ultrasound and provide the most comprehensive assessment of the major inspiratory muscles.

In this study, the ICCs between the two observers using different workstation software were high for diaphragm volume measurements on 3D-CT. We considered the use of only echography to measure muscle thickness as insufficient for evaluating the diaphragm. Rather, we expect the present measurement method for evaluating the morphology and muscle volume of the diaphragm to be more useful when determining the timing of postoperative extubation. Diaphragmatic assessment is also commonly used in other areas of research such as in sarcopenia, physical development, and aging, where various situations and conditions can affect the ability of the diaphragm muscles to generate force. Although the diaphragm muscles can significantly weaken with age, leading to dysfunction, it is unclear whether there are any specific correla-

tions between aging and the diaphragm.17) Total diaphragm volume evaluation by a new measurement method could be useful in clarifying any such relationships. Furthermore, in patients with a disease of the diaphragm, morphological abnormalities due to aging or disuse, and decreased muscle volume, aggressive respiratory rehabilitation is expected to improve diaphragm function, treatment results, and quality of life. In the future, this method could be used to evaluate respiratory function in surgical treatment and ICU patients. To avoid the issue of additional radiation exposure, CT data obtained during preoperative evaluations could be used.

This study has some limitations, including its small sample size (five subjects). However, it was possible to evaluate the diaphragm volume in all cases. Furthermore, the proposed measurement method involves radiation exposure, and it is still unknown whether the measured volume reflects the actual volume of the diaphragm. Additionally, technical standardization is necessary. Finally, these results may not be generalizable to other populations.

In conclusion, this study is the first to standardize the method for measuring the total diaphragm volume and examine the reproducibility and validity of the new method. The diaphragm could be selectively extracted and reconstructed. Measurement of the total diaphragm muscle volume using a workstation to reconstruct a stereoscopic image is feasible and highly reproducible. This technique can be reliably employed to evaluate diaphragm volume, thickness, and morphology.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article.

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Author contributions

All authors contributed to the conception of study design and data acquisition. AA, TM (TMorita), and TM (TMori) analyzed and interpreted the data and critically revised the manuscript for important intellectual content. AA was responsible for the investigation, methodology, drafting of the manuscript, validation, and visualization. TM (TMorita) was responsible for the project administration. AA (AAmano) critically revised the manuscript for important intellectual content and supervised the study. 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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Appearance and Frequency of Deep Venous Thrombosis After Total Hip Arthroplasty

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Objectives: Postoperative venous thromboembolism is an important peri-operative complication associated with total hip arthroplasty (THA). In particular, early detection of deep venous thrombosis (DVT) is important for the prevention of pulmonary embolism (PE). However, the methods and timing of examinations for DVT detection differ among the facilities. This study aimed to clarify the time, site, and frequency of DVT after THA.

Materials and Methods: Background characteristics including age, sex, body mass index, diagnosis, operation type, operation time, and anesthesia type were investigated in 348 patients who underwent THA at our hospital between April 2017 and April 2019. Blood tests were performed preoperatively and on postoperative days (POD) 0, 1, 3, 7, 14, and 21. Simultaneously, vascular ultrasonography was performed to investigate the time and site of thrombus occurrence before and after the surgery.

Results: DVT was observed in 27.2% of the patients on POD 21. The DVT-positive ratio was 9.4% (6/64) in males and 31.3% (89/284) in females. There was a significant difference between the groups ($p = 0.0002$). Patients in the DVT-positive group were significantly older than those in the DVT-negative group (73.0 ± 7.9 years vs. 63.3 ± 11.2 years, $p = 0.0041$). DVT mainly occurred in the soleal vein (74.7%). However, there was no significant difference between the operated and non-operated sides. In the DVT-positive group, thrombus occurred in 13.3% of preoperative cases, 20.0% on POD 0, 46.7% on POD 1, 13.3% on POD 3, 6.7% on POD 7, and 0% on POD 14 and 21.

Conclusions: Vascular ultrasonography showed that thrombus occurred most frequently in the soleal vein. Thrombus occurred in 66.6% of DVT-positive patients by POD 1, indicating that thrombus appeared very early after surgery. All thrombus cases were formed by POD 7.

Key words: deep venous thrombosis (DVT), pulmonary embolism (PE), ultrasonography, total hip arthroplasty (THA)

Introduction

Owing to the current trend in aging societies, the number of total hip arthroplasty (THA) operations will increase in the future. THA is associated with various peri-operative complications, among which postoperative venous thromboembolism (VTE) is particularly important.

VTE combines deep venous thrombosis (DVT) and pulmonary embolism (PE). DVT is caused by thrombus formation in deep veins such as the iliac, femoral, popliteal, and lower leg veins. Thrombus release from the deep veins can be considered a cause of PE. After release, the thrombus flows into the bloodstream, passes through the right atrium and ventricle, and embolizes the pulmonary artery.

The frequency of DVT diagnosed by venography without prophylaxis in patients who have undergone THA was estimated to be 40–60% in the 7th American College of Chest Physicians (ACCP)

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Guidelines[1] and 30–50% in the Japanese prevention guidelines[2]. According to the 7th ACCP Guidelines, postoperative symptomatic PE and fatal PE occurred in 1–30% and 0.1–7.5% of cases, respectively[1], compared to approximately 1% and 0.1% of cases, in the Japanese prevention guidelines[2].

The early detection of DVT is important for the prevention of PE because PE can lead to fatal complications. However, the methods and timing of examinations for DVT detection differ among the facilities. This study aimed to investigate and clarify the time, site, and frequency of DVT occurrences after THA.

Materials and Methods

Written informed consent was obtained from all participants, and the study protocol was approved by the appropriate ethics committee (approval number: 17–37). Background characteristics such as age, sex, body mass index (BMI), diagnosis, type of operation, operation time, and type of anesthesia were investigated in 383 patients who underwent THA at the Juntendo Nerima Hospital between April 2017 and April 2019. Patients with rheumatism, use of preoperative anticoagulants, female hormones or steroids, and a history of VTE were subsequently excluded.

Table 1 shows the inspection flowchart. Blood tests of the coagulation system, such as D-dimer and fibrinogen degradation products (FDP), and vascular ultrasonography (US) of the lower limbs were performed preoperatively and on postoperative days (POD) 0, 1, 3, 7, 14, and 21 to investigate the time and site of thrombus occurrence before and after surgery.

The US of the lower extremities was performed using a whole-leg US method, and we were able to observe the femoral and popliteal veins continuously using a compression method. Subsequently, the posterior tibial, peroneal, soleal, and sural veins were carefully observed. Visualizing the veins with a probe, we defined cases in which there was no obstruction of the lumen of the vein by compression of the probe and the blood flow signal was missing in the color Doppler as a positive finding. Furthermore, we classified patients as DVT-positive if they had at least one DVT positive finding by US between preoperative and POD 21, and DVT-negative if they had no DVT positive findings. The presence of PE was examined using contrast-enhanced computed tomography (CT) or pulmonary perfusion scintigraphy on POD 7 for all patients.

Continuous variables are summarized as means (standard deviation). Normally distributed continuous data sets were analyzed using Student's two-sample t-test. Categorical data was analyzed using Fisher's exact test. All the tests were two-sided, and a p-value < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad PRISM Version 7.03 (GraphPad Software, 2365 Northside Dr. Suite 560, San Diego, CA 92108, USA).

Results

We examined the occurrence of DVT using whole-leg US after THA in 383 patients from April 2017 to April 2019. For the present study, 35 patients were excluded because of rheumatics (n = 4), use of preoperative anticoagulants (n = 23), use of steroids (n = 3), a history of thrombosis (n = 5), and none of the patients used female hormones.

The remaining patients comprised 64 men with a mean age of 53.5 ± 10.4 years (range, 39–73 years) and 284 women with a mean age of 67.5 years (range, 48–90 years). The diagnoses were osteoarthritis in 330 cases and osteonecrosis (non-steroid) in 18 cases. The mean operation time

Table 1 Flow chart of patient background surveys and blood sampling/ultrasound examinations

Perioperative	Pre	POD 0	POD 1	POD 3	POD 7	POD 14	POD 21
Patient background	●						
Blood Test	●	●	●	●	●	●	●
Contrast enhanced CT or pulmonary perfusion scintigraphy					●		
Ultrasonography	●	●	●	●	●	●	●

POD, post-operative day

was 130.1 ± 34.9 min (range, 115–158 min). All surgeries were performed under general anesthesia. Cementless implants were used in all patients (Table 2).

DVT formation was observed until POD 21 in 95 patients (27.2%). No thrombus was observed in 253 patients (72.8%). The DVT-positive ratio was 9.4% (6/64) in men and 31.3% (89/284) in women. There was a significant difference between the groups (p = 0.0002). The mean age in the DVT-positive group was 73.0 ± 7.9 years (range, 55–84 years), while that in the DVT-negative group was 63.3 ± 11.2 years (range, 39–90 years). Patients in the DVT-positive group were significantly older than those in the DVT-negative group (p = 0.0041).

We did not observe any patients with symptomatic PE. The mean BMI in the DVT-positive group was 22.4 ± 2.2 kg/m^2 (range, 18.0–26.2 kg/m^2), while that in the DVT-negative group was 22.9 ± 2.9 kg/m^2 (range, 17.6–24.9 kg/m^2), with no significant difference (p = 0.2956). The mean operating time was 135.1 ± 41.0 min (range, 93–154 min) in the DVT-positive group and 125.9 ± 20.03 min (range, 104–171 min) in the DVT-negative group, with no significant difference (p = 0.4509). In our hospital, all patients were administered enoxaparin at 2,000 units twice a day for two weeks after surgery and an intermittent pneumatic compression device during the perioperative period for thromboprophylaxis. The thrombus was diagnosed as the distal type in 89 patients (93.7% of the DVT-positive group). Thrombus occurred in the soleal vein in 71 patients (74.7% of the DVT-positive group), but there was no significant difference between the operated and non-operated sides. The thrombus in the popliteal vein (proximal type) was observed in 6 patients (6.3% of the DVT-positive group) (Table 3).

Postoperative changes in D-dimer values are shown in Figure 1. At POD 3 and 7, the D-dimer value was significantly higher in the DVT-positive group compared with the DVT-negative group (POD 3: 6.08 μg/ml in the positive group vs. 4.39 μg/ml in the negative group, p = 0.0014; POD 7: 10.65 μg/ml in the positive group vs. 8.79 μg/ml in the negative group, p = 0.021).

The postoperative changes in FDP values are shown in Figure 2. There was no significant difference in the FDP values between the DVT-positive and negative groups.

The changes in DVT-positive rates are shown in Figure 3. The DVT-positive rates were 3.0% preoperatively, 9.0% on POD 0, 20.0% on POD 1, 18.1% on POD 3, 18.1% on POD 7, 16.3% on POD 14, and 16.3% on POD 21.

Figure 4 shows the time points at which DVT was first observed. In the DVT-positive group, the frequencies for the first observation of DVT were

Table 2 Demographic and surgical characteristics of the patients

	Male	Female	Over-all
No. of patients	64(18.4%)	284(81.6%)	348
Age(years)	53.5±10.4(39–73)	67.5±10.4(48–90)	66.0±11.2
BMI (%)	25.7±2.1(21.8–32.8)	22.3±4.2(17.6–24.7)	22.8±2.75
Diagnosis			
Osteoarthritis	52	278	330(94.8%)
Osteonecrosis	12	6	18(5.2%)
Others	0	0	0
Type of operation			
Primary	64	284	348
Revision	0	0	0
Operation time(min)	132(115–153)	129.9(93–158)	130.1±34.9
Type of anesthesia			
General	64	284	348
Spinal	0	0	0

BMI, body mass index

Table 3 Characteristics of patients in the DVT-positive group

	DVT+	DVT-
No. of patients (total 348)	95 (27.2%)	253 (72.8%)
Male (64)	6 (9.4%)	58 (90.6%)
Female (284) (P=0.0002)	89 (31.3%)	195 (68.7%)
Age (years) (P=0.041)	73.0±7.9 (55-84)	63.3±11.2 (39-90)
Operation time (min) (P=0.4509)	135.1±41.0 (93-154)	125.9±20.3 (104-171)
BMI (%) (P=0.2956)	22.4±2.2 (18.0-26.2)	22.9±2.9 (17.6-24.7)
Deep Venous Thrombosis		
Proximal	6 (6.3%)	-
Distal	89 (93.7%)	-
Symptomatic PE	0	-
Side of VTE		-
Operated side	39 (41.0%)	-
Non-operated side	44 (46.1%)	-
Both	12 (12.8%)	-

BMI, body mass index; DVT, deep venous thrombosis; PE, pulmonary embolism, Proximal= iliac-popliteal vein, Distal=lower leg vein

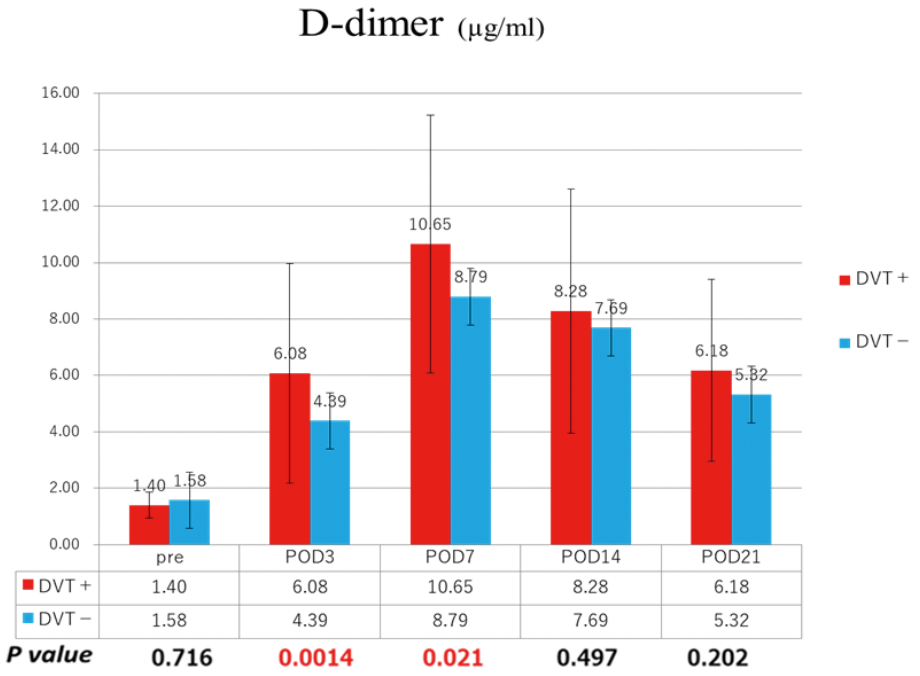

Figure 1 Changes in D-dimer values
On POD 3 and POD 7, the D-dimer value was significantly higher in the DVT-positive group compared with the DVT-negative group (POD 3: 6.08 μg/ml in positive group vs. 4.39 μg/ml in negative group, P=0.0014; POD 7: 10.65 μg/ml in positive group vs. 8.79 μg/ml in negative group, P=0.021). POD, post-operative day; DVT, deep venous thrombosis

20.0% on POD 0, 46.7% on POD 1, 13.3% on POD 3, 6.7% on POD 7, and 0% on POD 14 and 21.

Discussion

THA is a surgical procedure associated with a high risk of DVT. Therefore, physical and drug prophylaxis is recommended for THA performance. In our hospital, all patients were administered enoxaparin for thromboprophylaxis at 2,000 units twice a day for two weeks after surgery.

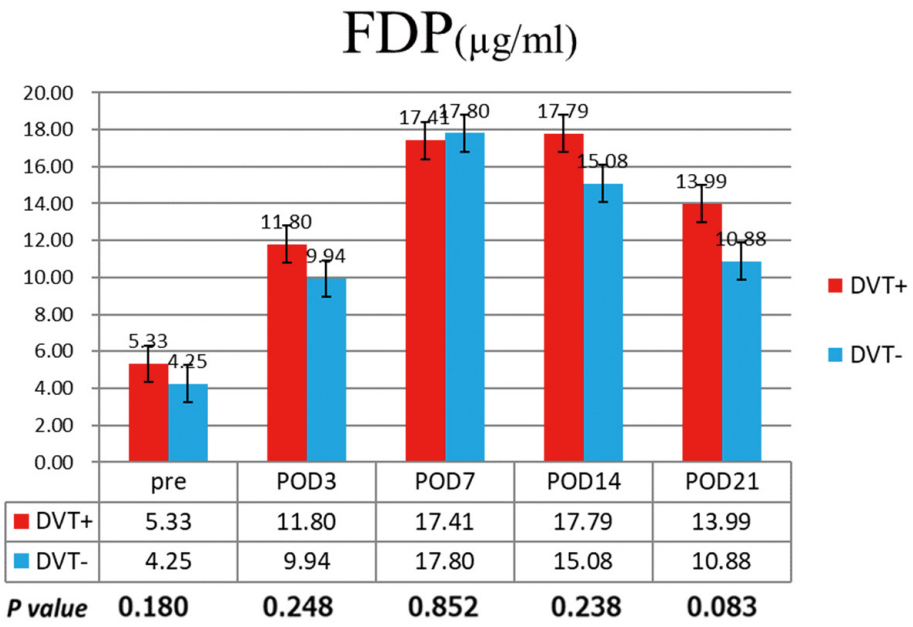

Figure 2 Changes in FDP values
There was no significant difference in FDP values between the DVT-positive group and the DVT-negative group. DVT, deep venous thrombosis; FDP, fibrinogen degradation products

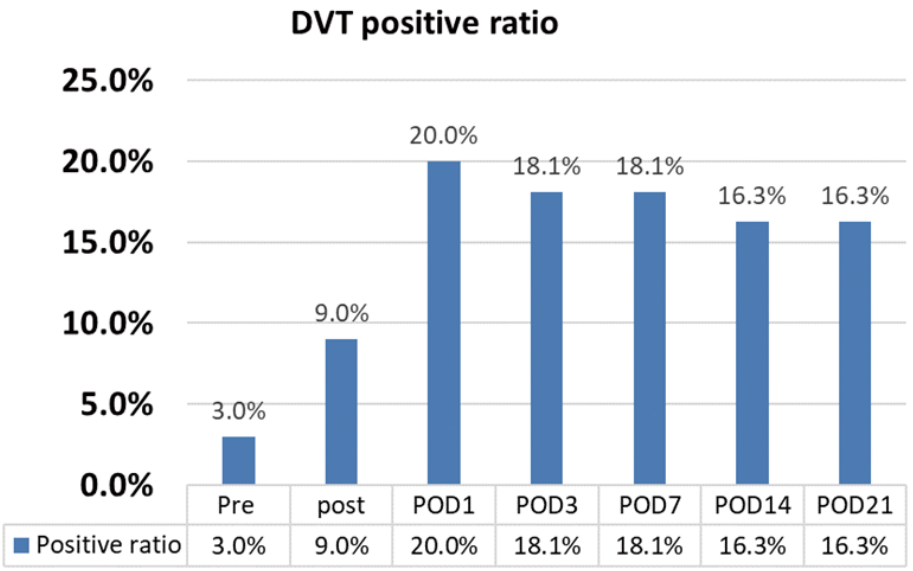

Figure 3 DVT-positive rates
The DVT-positive rates in all cases were 3.0% pre-operatively, 9.0% on POD 0, 20.0% on POD 1, 18.1% on POD 3, 18.1% on POD 7, 16.3% on POD 14, and 16.3% on POD 21. POD, post-operative day; DVT, deep venous thrombosis

Despite thromboprophylaxis, we observed DVT in 27.2% of the cases. This incidence of DVT was less than the rates of 34–63% in previous reports[3–9]. In this study, thrombus mostly formed in the soleal vein, consistent with previous reports[10, 11].

In previous studies, the majority of thrombus cases were found in the lower leg and disappeared within a few days, but approximately 30% extended to the proximal side within a few weeks[12–14]. Thrombus that develops on the proximal side tends to become free-floating within a short period, leading to widespread pulmonary thromboembolism[15]. Therefore, the source of embolization for severe PE is often proximal to the popliteal vein, particularly the femoral vein. However, it can sometimes occur on the distal side[11, 15, 16], and from the

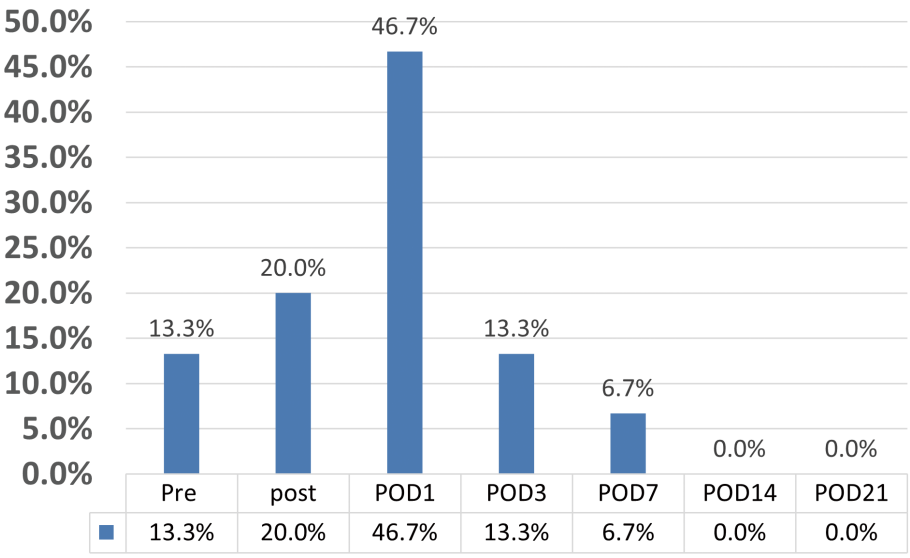

Figure 4 Time points when thrombus became positive for the first time in the DVT-positive group
In the DVT-positive group, the frequencies for first observation of DVT were 20.0% on POD 0, 46.7% on POD 1, 13.3% on POD 3, 6.7% on POD 7, and 0% on POD 14 and POD 21.
POD, post-operative day; DVT, deep venous thrombosis

soleal veins[11]. Therefore, for distal thrombosis with a tendency towards enlargement, it is necessary to check for thrombus at regular intervals.

Regarding imaging diagnosis, noninvasive venous US is the first choice for thrombosis detection in the lower extremities because of high diagnostic accuracy[17-19]. Contrast-enhanced CT examination involves administration of a contrast agent and radiation exposure. We sometimes encountered rare cases in which clinical VTE was highly suspected without positive US findings. In such cases, contrast-enhanced CT or magnetic resonance imaging should be performed[18, 20-22].

US can continuously visualize the femoral, popliteal, and lower leg veins in B mode. A compression US technique was used, in which the vein was compressed with a probe, allowing the determination of the presence or absence of a thrombus. Color, power, and pulse Doppler methods are commonly used to visualize blood vessels and check for obstructions[18, 23, 24]. The mean sensitivity for proximal-type DVT in the femoral and popliteal veins, compared with venography, was reported to be 97% (range, 89-100%) for symptomatic patients and 62% (range, 38-100%) for asymptomatic patients[25]. Furthermore, the mean sensitivity of US for distal-type DVT was 73% (range, 0-100%) for symptomatic patients and 53% (range, 0-92%) for asymptomatic patients[25]. However, the specificity compared with venography was 96% (95% CI, 95.2-96.8%)[18]. The result of 27% of DVT positive cases in our study may be lower than the actual result because the sensitivity of US is low in the distal-type. However, since the specificity is high, there is little possibility that false positives are included.

In the present study, the patients in the DVT-positive group were significantly older than those in the DVT-negative group. The risk of DVT was reported to gradually increase every 10 years with age, and the risk of DVT in patients aged >65 years was 2.1 times higher[26, 27]. The results relative to the mean age in the present study correspond to those of previous studies. In addition, the DVT-positive ratio was 9.4% (6/64) in men and 31.3% (89/284) in women. There was a significant difference between the groups (p = 0.0002). The factors that cause DVT more frequently in women than in men may be that the average age of women is older than that of men and that older people are more likely to delay rehabilitation progress[28, 29]. In addition, it is possible that lower preoperative activities of daily living due to advanced age may affect postoperative walking ability[28, 29], and lower limb pumping ability due to differences in muscle mass may contribute to venous stasis[30].

In the DVT-positive group, thrombus formation was observed in 66.6% of cases by POD 1, indicating that the thrombus appeared very early after surgery. This finding may be reflected by the high D-dimer value observed during the early postoperative period. Furthermore, body posture during surgery and bed rest until POD 1 may facilitate thrombus formation. However, there was no significant difference in the operation time between the DVT-positive and DVT-negative groups. In addition, there were no significant differences between the operated and non-operated sides. The affected femur and lower leg were always grasped by the assistant, and the limb position was continuously moved according to the surgical situation. Therefore, it is possible that the massage effect was equivalent to that on the healthy side to which the intermittent pneumatic compression device was attached. It is also possible that postoperative rehabilitation may be affected by the early start of range of motion training on the affected side. Regarding the operation, the duration of vein occlusion in the affected hip joint with flexion and the internal rotation position at the time of implant insertion may be intraoperative factors. However, we did not examine these factors in this study. Nevertheless, we can conclude that a distal thrombus formed very early after the operation. Moreover, we can clarify that many thrombus cases after THA were found by POD 1, and almost all thrombus formation was completed by POD 7.

Attention should be paid to PE because its results are serious. Nakamura et al.[31] investigated 108 acute PE patients and reported that 57% developed symptoms while standing or walking and 22% developed symptoms after defecation or urination. Therefore, we need to pay careful attention to the physical condition of patients when they get out of bed, depending on the tendency for increased thrombus formation. In high-risk patients, it is necessary to check for thrombus formation using US to prevent symptomatic PE preoperatively and when they get out of bed. Therefore, as we did, it is better to perform blood sampling and a lower limb US test during the perioperative period and to perform contrast-enhanced CT for positive individuals. In this study, the previously mentioned prevention did not cause symptomatic PE in all subjects except the high-risk group.

A limitation of this study is that US was performed by one person, and hence, the inter-rater reliability cannot be calculated accurately.

In conclusion, the majority of thrombus cases were found on POD 1, and thrombus formation was mostly completed by POD 7. The thrombus formed very early after the surgery, indicating that US should be performed as soon as possible during the perioperative period. In patients who have a tendency for thrombus formation postoperatively, it may be necessary to examine thrombus formation.

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Author contributions

MN, SK, YS, HO, SK obtained informed consent from the patients and performed total hip arthroplasty and various tests for research. MI supervised this study and provided advice and guidance. All authors read and approved the final manuscript.

Conflicts of interest statement

Authors declare that there are no conflicts of interest.

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Original Articles

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Development of a Non-invasive Diagnostic Method for Esophageal Squamous Cell Carcinoma by Gas Chromatographic Analysis of Exhaled Breath

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Objectives: Since esophageal carcinoma progresses asymptomatically, for many patients the disease is already advanced at the time of diagnosis. The main methods that are currently used to diagnose esophageal carcinoma are upper gastrointestinal radiographic contrast examinations and upper gastrointestinal endoscopy, but early discovery of this disease remains difficult. There is a need to develop a diagnostic method using biomarkers that is non-invasive while both highly sensitive and specific.
Materials and Methods: Exhaled breath was collected from 17 patients with esophageal squamous cell carcinoma (ESCC), as well as 9 control subjects without history of any cancer. For each fasting subject, 1L of exhaled breath was collected in a gas sampling bag. Volatile organic compounds (VOCs) were then extracted from each sample using Solid phase micro-extraction (SPME) fibers and analyzed by gas chromatography-mass spectrometry (GC-MS).
Results: Levels of acetonitrile, acetic acid, acetone, and 2-butanone in exhaled breath were significantly higher in the patient group than in the control group ($p = 0.0037$, 0.0024, 0.0024 and 0.0037, respectively). ROC curves were drawn for these 4 VOCs, and the results for the area-under-the-curve (AUC) indicated that ESCC patients can be identified with a high probability of 0.93.
Conclusion: We found distinctive VOCs in exhaled breath of ESCC patients. These VOCs have a potential as new clinical biomarkers for ESCC. The measurement of VOCs in exhaled breath may be a useful, non-invasive method for diagnosis of ESCC.

Key words: Volatile organic compounds (VOCs), Esophageal squamous cell carcinoma (ESCC), Gas chromatography-mass spectrometry (GC-MS)

Introduction

Esophageal carcinoma is the tenth most common malignancy in the world[1)], and the tenth most common cause of cancer death in Japan[2)]. Incidence of squamous cell carcinoma of the esophagus (ESCC) is decreasing in Europe and America, whereas the incidence of esophageal adenocarcinoma is, conversely, trending upward. In Asia, squamous cell carcinoma still accounts for a majority of all esophageal carcinomas[3)]. Known risk factors for ESCC include smoking, drinking alcoholic beverages, hot foods, drinks and malnutrition[4)]. The quality of diagnosis and treatment has improved recently, but these cancers still have a poor prognosis. The main symptoms of esophageal carcinoma are dysphagia and a feeling that food is stuck in the throat. However, because only advanced cancers cause these symptoms, there are usually no symptoms in the early stage of the disease. As a result,

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discovery of esophageal carcinomas in its early stage remains difficult. Clinical biomarkers and tests for early detection of esophageal carcinomas have not yet been established. Developments of non-invasive method for early diagnosis with better sensitivity and specificity are expected.

In recent decades, various scent detection studies have been performed with animals and electronic devices[5]. The presence of some infections and malignant tumors can be identified based on changes in patients' metabolites. Devices for detecting volatile organic compounds (VOCs) that are odorous elements of cancers are also being developed[6, 7]. McColloch et al. reported high accuracy rates using the exhaled breath of dogs with lung and breast cancers[8]. Breath analysis using gas chromatography has also identified several VOCs specific to lung and breast cancers[9-11]. The identification of cancers by exhaled breath analysis is expected to develop as non-invasive methods for detection of early-stage cancer. Recent studies demonstrated the clinical potential of VOCs profiling to identify esophageal or gastric adenocarcinoma[12, 13]. To date, there have been no reports of VOCs specific to ESCC, and the present pilot study aimed to identify just such VOCs as clinical biomarkers.

Materials and Methods

Subjects

Exhaled breath was collected from 17 patients who had been diagnosed with ESCC and 9 healthy volunteers in Juntendo University Hospital between July 2012 and November 2013. Healthy volunteers were selected from staffs at our facility who had been approved to participate in the study and who had no history of cancer or other medical conditions. No upper gastrointestinal endoscopy was required. The exhaled breath was analyzed for its VOC contents. All subjects were interviewed to confirm the disease for which they were being treated, whether they were taking any medications or smoked, and their family histories. Patients who were being treated with either radiotherapy or chemotherapy were excluded from the study, since such treatments might alter their metabolism. All protocols were approved by the ethical committee of the Juntendo University Hospital (No.16-062), and all participants provided written informed consent before their participation in this study according to the guidelines established in the Declaration of Helsinki.

Exhaled breath collection

All participants were fasted for 6-8 hours prior to their breath sample collection. For each subject, 1L of exhaled breath was collected in a Supel™ Inert Gas Sampling Bag (Sigma-Aldrich Co. LLC. MO, USA) in a clean-air environment before any treatment. Prior to the collection, each sampling bag was washed with nitrogen to reduce other influences. All collected samples were stored and processed in a cold room at 4℃.

Extraction and analysis of volatile organic compounds

Samples were analyzed by gas chromatography-mass spectrometry (GC-MS) combined with solid phase micro extraction (SPME) (Sigma-Aldrich Co. LLC. MO, USA). The SPME was used for pre-concentration of VOCs in the breath samples. A manual SPME holder with 85μm Carboxen®/Polydimethylsiloxane (CAR/PDMS) fiber (Sigma-Aldrich Co. LLC. MO, USA) was inserted into the Supel™ Inert Gas Sampling bags for 16 hours at room temperature. The VOCs extracted in SPME fiber were desorbed thermally in the heated GC injector at 250°C (splitless mode) and analyzed by GC-MS. The GC-MS analysis was performed with a TRACE GC connected to TSQ QUANTUM GC (Thermo Fisher Scientific Inc., MA, USA). A CP Pora PLOT Q capillary column (0.25mm i.d x 25m x 8μm firm thickness, Agilent Technologies Inc, CA, USA) was used for separation of VOCs. The column temperature started at 40°C for 3 minutes, then raised at 10°C per minute to 250°C and held for 5 minutes. Helium was used as carrier gas at 1ml/min. MS transfer line was set at 250°C. The MS analysis was carried out Electron impact ionization (EI) mode with 70eV of electron energy at scan range m/z:10-400. Ion source temperature was set at 250°C. The detected VOCs were identified by NIST MS Search 2.0 (Thermo Fisher Scientific Inc., MA, USA).

Statistical analysis

After alignment the comprehensive peaks in GC-MS chromatograms of every sample using Thermo Scientific SIEVE Software (Thermo

Fisher Scientific Inc., MA, USA), the Fisher Ratio was calculated against individual frames and significant peaks were extracted to find candidate for biomarkers which discriminated 2 groups[14]. VOCs were identified by comparing EI mass spectra of each peak with that of the NIST Mass Spectral Library Version 2.0f using NIST MS Search 2.0. The area of selected ion peak in each candidate was applied to principal component analysis (PCA). And those of them that were significantly higher in the patient group than in the healthy volunteer group were used to generate receiver-operating characteristics (ROC) curves using the free ROC analysis software (WROCFIT). Statistical analysis was performed using *t*-test. The differentially expressed compounds with p-values of < 0.05 were considered statistically significant. Receiver-operating characteristics (ROC) curve was used to evaluate the discriminatory power of selected VOCs for differentiation of patients and controls.

Results

Study population

The clinical characteristics of the patient group are summarized in Table 1. Seventeen esophageal carcinoma patients and 9 volunteer control subjects were enrolled in this study. All subjects in the patient group had been pathologically diagnosed with advanced ESCC.

VOCs profiling of samples

Representative GC/MS total ion chromatograms of samples from the patient group and the control group were showed in Figure 1. Statistical analysis of the obtained peaks revealed that 15 VOCs containing 4 VOCs, i.e. acetonitrile, acetic acid, acetone, 2-butanone, of which were significantly higher in the patient group than in the healthy volunteer group.

Figure 2 showed differences of peak area for 4 VOCs in two groups. These differences were found to be significant (acetonitrile, $p = 0.0037$; acetic acid, $p = 0.0024$; acetone, $p = 0.0024$; 2-butanone, $p = 0.0037$).

ROC curves were drawn for acetonitrile, acetic acid, acetone, and 2-butanone. The results for the area-under-the-curve (AUC) of the combination of these 4 VOCs indicated that ESCC patients can be

Table 1 Clinical characteristics of esophageal carcinoma patients in this study

Patient no.	Sex	Age	Smoking	Family history of cancer	Histology[a]	Main location of the lesion[b]	Size (mm)	Differentiation[c]	Depth of tumor invasion	Lymph node metastasis	Distant metastasis	Stage
1	M	40	Yes	No	SCC	CeUt	50	WD	T3	N2	No	ⅢB
2	F	66	No	No	SCC	Lt	70	MD	T3	N2	No	ⅢB
3	M	70	Yes	No	SCC	Ce	45	PD	T3	N3	No	ⅢC
4	M	66	Yes	Yes	SCC	Lt	44	MD	T3	N0	lung	Ⅳ
5	M	55	No	Yes	SCC	MtUt	42	MD	T4b	N2	No	ⅢB
6	M	55	Yes	Yes	SCC	Mt	36	MD	T3	N2	No	ⅢB
7	M	67	Yes	Yes	SCC	CeUt	50	MD	T3	N2	No	ⅢB
8	M	66	Yes	Yes	SCC	Mt	39	PD	T2	N2	No	ⅢB
9	M	81	Yes	No	SCC	MtUt	66	MD	T4b	N3	No	ⅢC
10	M	55	Yes	Yes	SCC	Ut	30	PD	T2	N2	No	ⅢB
11	M	62	Yes	Yes	SCC	LtAe	45	MD	T3	N1	No	ⅢA
12	M	70	Yes	Yes	SCC	CeUt	80	MD	T3	N3	No	ⅢC
13	M	65	Yes	Yes	SCC	CeUt	60	MD	T4b	N3	No	ⅢC
14	F	65	Yes	No	SCC	LtMt	76	MD	T3	N3	No	ⅢC
15	M	68	Yes	No	SCC	MtLt	60	MD	T3	N2	No	ⅢB
16	F	83	Yes	No	SCC	Ce	20	MD	T4b	N2	No	ⅢC
17	F	80	No	Yes	SCC	Mt	25	MD	T3	N1	No	ⅢA

[a] SCC, esophageal squamous cell carcinoma

[b] Ce, cervical esophagus; Ut, upper thoracic esophagus; Mt, middle thoracic esophagus; Lt, lower thoracic esophagus; Ae, abdominal esophagus

[c] WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated

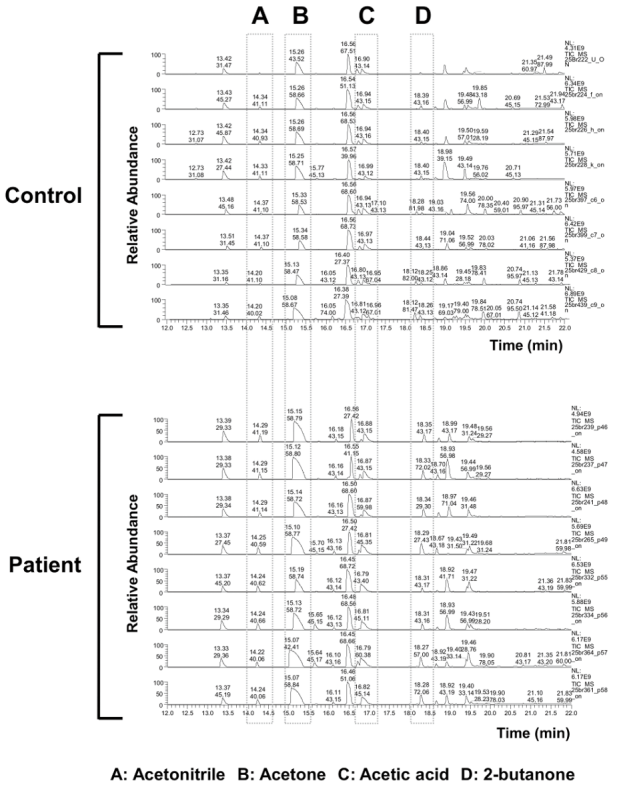

Figure 1 Representative GC/MS total ion chromatograms of the breath samples from the control group and the patient group
Each peak shows (A) acetonitrile, (B) acetic acid, (C) acetone, and (D) 2-butanone.

identified with a high probability of 0.93 (Figure 3).

Discussion

There was significant difference in VOCs included in exhaled breath between the ESCC patient group and the control group: acetone, acetic acid, acetonitrile, and 2-butanone showed higher concentration in the patient group. To the best of our knowledge, this is the first report that demonstrates the significance of VOCs measurement in ESCC.

Recent years, several VOCs detection by breath analysis with GC/MS have been reported to be useful to identify lung and breast cancers[8–10]. Metabolomics studies for detecting various cancers are increasing[15–17]. However, studies that focus on ESCC are still relatively rare. Cancer growth is promoted by mutations in genes and proteins, leading to peroxidation of cell membranes and release of VOCs[18]. Endogenous cancer-specific VOCs are released from the cancer cells themselves and/or during the metabolic processes associated with cancer growth, and it is suspected that different substances and/or compounds with different molecular weights are released by different types of cancers. Moreover, it is not just that VOCs are

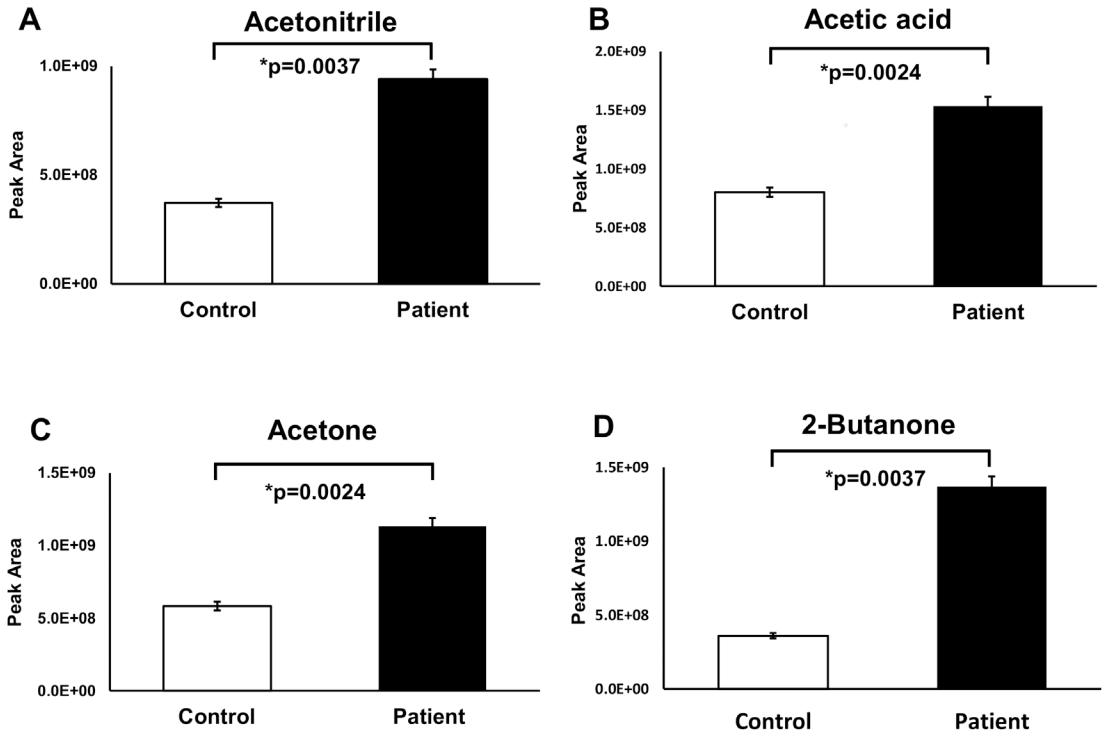

Figure 2 Comparison of the 4 VOCs level between in the control group and the patient group
The peak area of (A) acetonitrile, acetic acid, (B) acetone, (C) acetone, and (D) 2-butanone in the ESCC patient group were significantly higher than those in the control group ($p<0.01$, respectively).

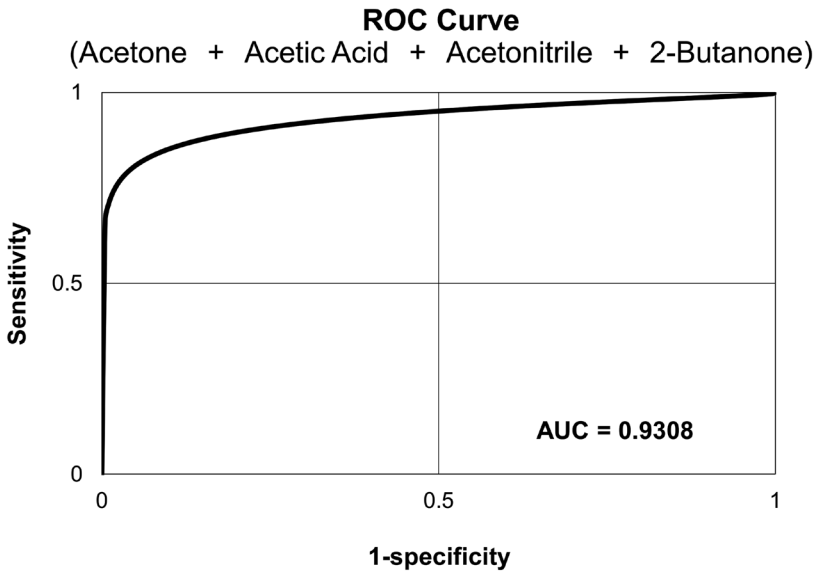

Figure 3 Receiver operating characteristic (ROC) curve for the detection of ESCC patients using the combination of acetonitrile, acetic acid, acetone, and 2-butanone concentration in exhalation
The area-under-the-curve (AUC) was 0.93.

released directly from cancers, but also that metabolites are conveyed from the blood stream by alveoli and enter the exhaled breath[19–21]. As a result, it is thought that it is possible to measure the changes in metabolism that are caused by cancer growth in exhaled breath[22].

In esophageal carcinoma patients, the exhaled breath is thought to include not only metabolites originating from the alveoli, but also many fragrant substances arising from the esophageal carcinoma itself. Thus, it is thought that breath analysis of VOCs would make it possible to identify substances specific to ESCC more accurately compared with other cancers. Altomare et al. found that 15 VOCs, including alkyl aldehydes, ketones, alkanes, and aromatic hydrocarbons, differed between healthy subjects and patients with colorectal cancer[23]. Huang et al. measured VOCs in urine samples and found differences in acetone, acetic acid, hexane, hydrogen sulfide, methanol, and phenol between patients with gastro esophageal cancer and healthy subjects[24]. Kumar et al. demonstrated that 12 VOCs in exhaled breath were present at significantly higher concentrations in the esophageal and gastric adenocarcinoma cancer groups than in the noncancer controls[13]. They reported that VOCs profiling was useful for early detection of cancers.

The present study has several limitations. First, this was a single center study with a small sample size, and it will be necessary to study a larger number of samples to confirm these findings. Second, the patients in this study had comparatively advanced cancers. There have been reports that lung, colorectal, and breast cancers could be distinguished from healthy individuals at a relatively early stage using this method[25–27]. Therefore, this method of cancer detection must be tested in patients with early-stage ESCC. Third, there are no established methods for breath analysis of VOCs, and there is a need to evaluate whether food, tobacco use, ingested drugs, concurrent diseases, genetic factors, etc., affect the results of this analysis method. Moreover, the composition of gastrointestinal microbiota could have a major impact on the exhaled breath of suspected digestive cancer patients; this factor has not yet been studied sufficiently in the context of diagnostic breath testing[28].

In conclusion, this study found that the levels of 4 VOCs, i.e., acetonitrile, acetic acid, acetone, and 2-butanone, were significantly higher in the exhaled breath of ESCC patients than in control subjects. Those findings indicate that measurement of VOCs in the exhaled breath has potential as a useful, non invasive method for diagnosis of ESCC.

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Author contributions

SM and YK made a study design, recruited patients and volunteers as control, collected data, analyzed, and interpreted the patient data and SM was a major contributor in writing the manuscript. NK, HT, TF, and TU performed the examination of the gas chromatography-mass spectrometry (GC-MS). TH and MN revised the article, and all authors 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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The Cingulate Island Sign is Useful for a Differential Diagnosis of Early-Onset Alzheimer's Disease and Dementia with Lewy Bodies: A 99mTc-ECD SPECT Study

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Introduction: Early-onset dementia is fast-progressing compared with late-onset dementia, with major clinical characteristics including prominent focal cerebral symptoms. Given its economic and psychological implications, proper diagnosis and treatment at an early stage is essential. In the present study, the authors conducted a retrospective study to evaluate the usefulness of various numerical indices (including CIScore calculated by eZIS, cerebral blood flow SPECT analysis software) in the differential diagnosis of early-onset dementia.

Materials and Methods: This study involved patients with early-onset and mild dementia who were receiving ambulatory care at our outpatient department specializing in Alzheimer's disease (14 MCI patients, 16 AD patients, and 16 probable/possible DLB patients). ROC analysis was performed for each SVA numerical index calculated by eZIS to calculate AUC. For the AD and DLB groups, correlation between the CIScore and MMSE was assessed.

Results: When SVA-A (severity) was used to differentiate AD from MCI and DLB from MCI, the respective AUC values were 0.960 and 0.911. When CIScore was used to differentiate AD from DLB (threshold value: 0.225), the obtained AUC value was 0.941, and the accuracy, sensitivity, and specificity were 90.6%, 87.5%, and 93.7%, respectively. No significant correlation was observed between the MMSE and CIScore scores in these disease groups.

Conclusion: The results of this study have suggested that the SVA-A is a useful index for evaluating the conversion from MCI to either early-onset AD or DLB, and that the CIScore is useful for differentiating AD from DLB in both late-onset and early-onset dementia cases.

Key words: early-onset dementia, early diagnosis, differential diagnosis, SPECT

Introduction

Early-onset dementia is a term used to describe any form of dementia that develops in people aged 64 or below. Epidemiological research estimates that approximately 40 thousand people have this condition in Japan. The most common cause is vascular dementia (VD), which is followed by Alzheimer's disease (AD), head trauma, and dementia with Lewy bodies (DLB)[1)]. Compared with late-onset dementia, early-onset dementia is fast-progressing and clinically characterized by prominent focal cerebral symptoms. Given its economic and psychological implications, proper diagnosis and treatment at an early stage is essential.

Neuropsychological tests, such as Mini-Mental State Examination (MMSE)[2)], are the primary means of diagnosing dementia. However, recent

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advances in cerebral function measurement technology have made it practicable to employ brain imaging analysis as an auxiliary measure for more accurate diagnosis. Technologies that are typically utilized for brain imaging include magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT). Clinically, detecting minute brain atrophy, a characteristic symptom of early-stage AD, requires high skill and long time. With the aim of easily and quantifiably identifying atrophied lesions in the hippocampus and parahippocampal gyrus in MR imagery, the Voxel-Based Specific Analysis System for Alzheimer's Disease (VSRAD)[3] has been recently developed and is now being used in medical practice. However, patients with early-onset dementia often have hippocampus/parahippocampal gyrus atrophies that are fairly inconspicuous, and it is not uncommon for VSRAD-based indices to fall within the normal range. At the stage of mild cognitive impairment (MCI), which is considered to be the prodromal phase of AD, known cerebral blood flow findings include a decrease in blood flow in the parietal lobe, precuneus, and posterior cingulate gyrus. Since even AD patients tend to display high blood circulation in the precuneus and posterior cingulate gyrus, it is difficult to macroscopically detect a decrease from early stages. An easy Z-score imaging system (eZIS) was developed to compare the normal blood flow of healthy people with that of AD patients[4-6] and is now being used for aiding AD diagnosis.

Depressiveness, hallucinations, and delusions are frequently observed symptoms of DLB[7]. However, presenile DLB patients are often falsely diagnosed with depression and/or schizophrenia due to these diseases sharing similar symptoms[8]. Impaired cognitive functions and psychiatric symptoms derived from DLB can be effectively treated with donepezil[9]. Therefore, early diagnosis is more crucial for DLB than for AD because it can significantly affect the prognosis. Moreover, predispositions of DLB patients, such as high sensitivity of antipsychotics, mean that differential diagnosis is critical. In DLB, sugar metabolism and blood flow decrease in the occipital lobe. Additionally, 18F-FDG PET imaging reveals a cingulate island sign (CIS), a finding that sugar metabolism is relatively retained in the posterior cingulate gyrus in DLB as opposed to AD[10]. According to the Clinical Diagnostic Criteria for DLB (2017 Revised Edition), the CIS is regarded as one of the major biomarkers[11]. Furthermore, a recent study has reported that DLB patients exhibit CIS findings are in cerebral blood flow SPECT using 99mTc-ECD, as compared with AD patients[12]. Another study has demonstrated the high effectiveness of CIScore (a reference index using CIS in eZIS) in differentiating AD from DLB[13].

In the present study, we retrospectively evaluated the usefulness of various numerical indices calculated by eZIS (cerebral blood flow SPECT analysis software) in differentiating types of early-onset dementia.

Materials and Methods

The present study was approved by the Ethics Committee of Juntendo University Hospital (No. JHS 17-0017) and was conducted with the informed consent of the subjects and their families in writing.

The study involved patients with early-onset and mild dementia who were receiving ambulatory care at our outpatient department specializing in Alzheimer's disease. The patient characteristics are summarized in Table 1. The subjects comprised 14 MCI patients (11 men and 3 women), 16 AD patients (11 men and 5 women), and 16 probable/possible DLB patients (6 men and 10 women). The MCI patients were diagnosed in accordance with Petersen's Criteria 2004, were 51 to 69 years old (mean: 58.4; standard deviation 6.3), and their MMSE score ranged from 22 to 30 (mean: 27.4; standard deviation: 2.3). The AD patients were diagnosed in accordance with DSM-IV-TR, were

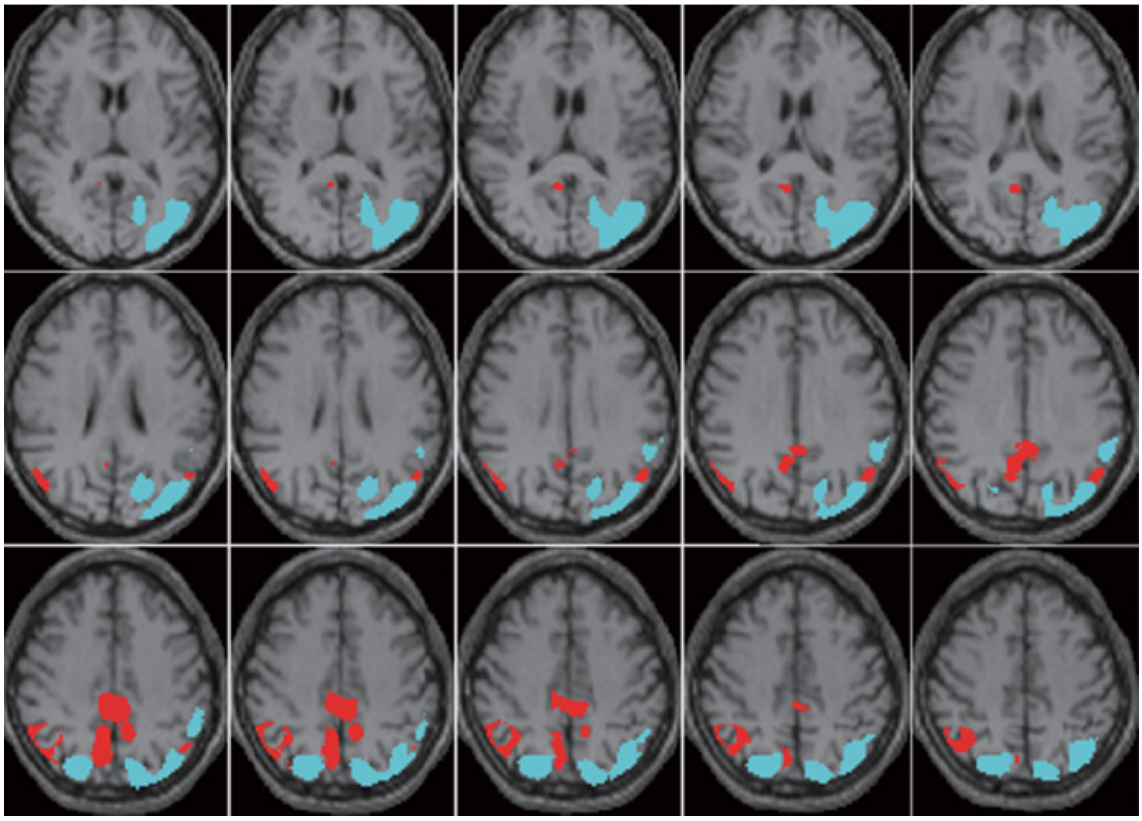
Table 1 Demographic data

Characteristic	MCI (n=14)	AD (n=16)	DLB (n=16)
Male / Female	11 / 3	11 / 5	6 / 10
Age (mean±SD)	58.4±6.3	58.3±5.7	61.6±3.8
MMSE score (mean±SD)	27.4±2.3	20.4±6.1	16.7±6.6

47 to 68 years old (mean: 58.3; standard deviation: 5.7), and their MMSE score ranged from 4 to 26 (mean: 20.4; standard deviation: 6.1). The DLB patients were diagnosed in accordance with the 3rd International WS DLB, were 54 to 69 years old (mean: 61.6; standard deviation: 3.8), and their MMSE score ranged from 5 to 26 (mean: 16.7; standard deviation 6.6).

All subjects underwent head SPECT at our Radiology Department. For SPECT, TOSHIBA GCA 9300 A, and SIEMENS Symbia_E, and S were used. Additionally, a fan beam collimator was used. A tracer (99mTc-ECD) was administered to all participants at a supine position, with the eyes closed. The parameters of SPECT imaging were as follows: matrix size: 128×128; step angle: 4 degrees; view: 90; rotation time: 1min; and rotation: 16.

Automatic analysis using eZIS (version 1.1.0) was performed to set a volume of interest (VOI). With eZIS, specific VOI analyses (SVA-A and SVA-B) were performed. The VOI of SVA-A is the area with significantly decreased blood flow ($p < 0.001$) in the 99mTc-ECD image of an incipient Alzheimer's dementia patient with mildly impaired cognitive functions (amnestic MCI), which is comparatively analyzed against the 99mTc-ECD image of a healthy person. The image includes the posterior and anterior cingulate gyrus and a part of the vertex. As numerical indices, severity (degree of blood flow decrease in the VOI), extent (percentage of area with decreased blood flow in the VOI), and ratio (ratio of area with decreased blood flow in the VOI and that in the entire brain) are calculated. The VOI of SVA-B is the area with significantly decreased blood flow ($p < 0.05$) in the 99mTc-ECD image of a DLB patient compared with that of a healthy person. When this area VOI1 (mainly the posterior head) is subtracted from the VOI of the aforementioned SVA-A VOI, the VOI2 (mainly the posterior cingulate gyrus) is set (visualization in Figure 1). The sum of the Z scores in the area with decreased blood flow obtained from these two VOIs are used to calculate the CIScore (CIScore = sum of the Z scores in the decreased blood flow area obtained from the VOI2/sum of the Z scores in the decreased blood flow area obtained



VOI1 (blue): Areas where blood flow is decreased in DLB compared to healthy person.
VOI2 (red): Areas where blood flow is relatively maintained in DLB compared to AD.

Figure 1 Examples of VOI1 and VOI2

from the VOI1). In a multi-center study so far, the normal Z score value has been reported as severity ≤ 1.19, extent 14.2 ≤ 2%, and ratio ≤ 2.22[14]. The MCI, AD, and DLB groups were analyzed by eZIS SVA calculate various numerical indices (severity, extent, ratio, and CIScore). Significant differences in the Z scores of the VOI1 and VOI2 were verified between the groups. Receiver operating characteristics (ROC) analysis was performed to calculate the area under the ROC curve (AUC). Additionally, the correlation between the CIScore and MMSE was assessed for the AD and DLB groups.

Results

Table 2 lists the numerical indices of all the groups, their means, and standard deviations. Mann-Whitney U test was performed for the numerical indices of each group, with $p < 0.05$ being the level of significance.

In the MCI group, all severity, extent, and ratio values exceeded the normal range. When the MCI group was compared with the AD and DLB groups, both the severity and extent values were significantly high, and the ratio value was not significantly different. Box plots of the Severity and the CIScore are shown in Figure 2. When severity was used for differentiating MCI from AD and DLB, the respective AUC values were 0.960 and 0.911. Likewise, when the CIScore and VOI1 were used, the respective AUC values were 0.186, 0.641, 0.785, and 0.988 (Figure 3). When the CIScore was used for differentiating AD from DLB, the obtained AUC was 0.941. When the threshold value was set at 0.225, the accuracy, sensitivity, and specificity were 90.6%, 87.5%, and 93.7% (Figure 4). When chronological changes of the SVA-A and CIScore in the MCI due to AD and MCI due to DLB cases were tracked, the chronological changes of the CIScore tended to decline (Figure 5). In all disease groups, no significant correlation between the CIScore and MMSE was observed (Figure 6).

Discussion

Compared with the MCI group, both the AD and DLB groups exhibited significantly increased severity and extent, and no significant difference was observed in the ratio. The obtained AUC also had high severity and extent, thus suggesting that severity and extent were useful indices for evaluating the conversion of MCI into other diseases in both the AD and DLB groups. The obtained AUC indicates that severity is more useful for differen-

Table 2 SVA index (mean±SD) in each group

	Severity	Extent	Ratio	CIScore	VOI-1	VOI-2
MCI	1.53†, ‡ ±0.33	26.27 ±13.80	4.29 ±2.40	0.227 ±0.130	8677 ±2478	1771 ±580
AD	3.38† ±1.31	68.72 ±20.41	5.84 ±2.73	0.358§ ±0.159	15159 ±7607	4826 ±2173
DLB	3.19‡ ±1.27	62.82 ±23.97	3.88 ±1.25	0.173§ ±0.057	24616 ±9125	4226 ±1971

†, ‡, §: significant difference

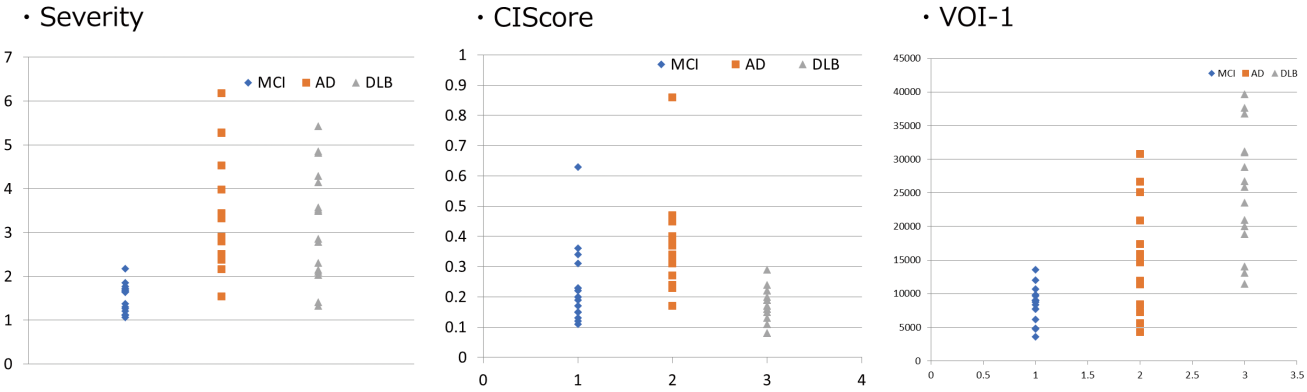


Figure 2 Boxplot in each group

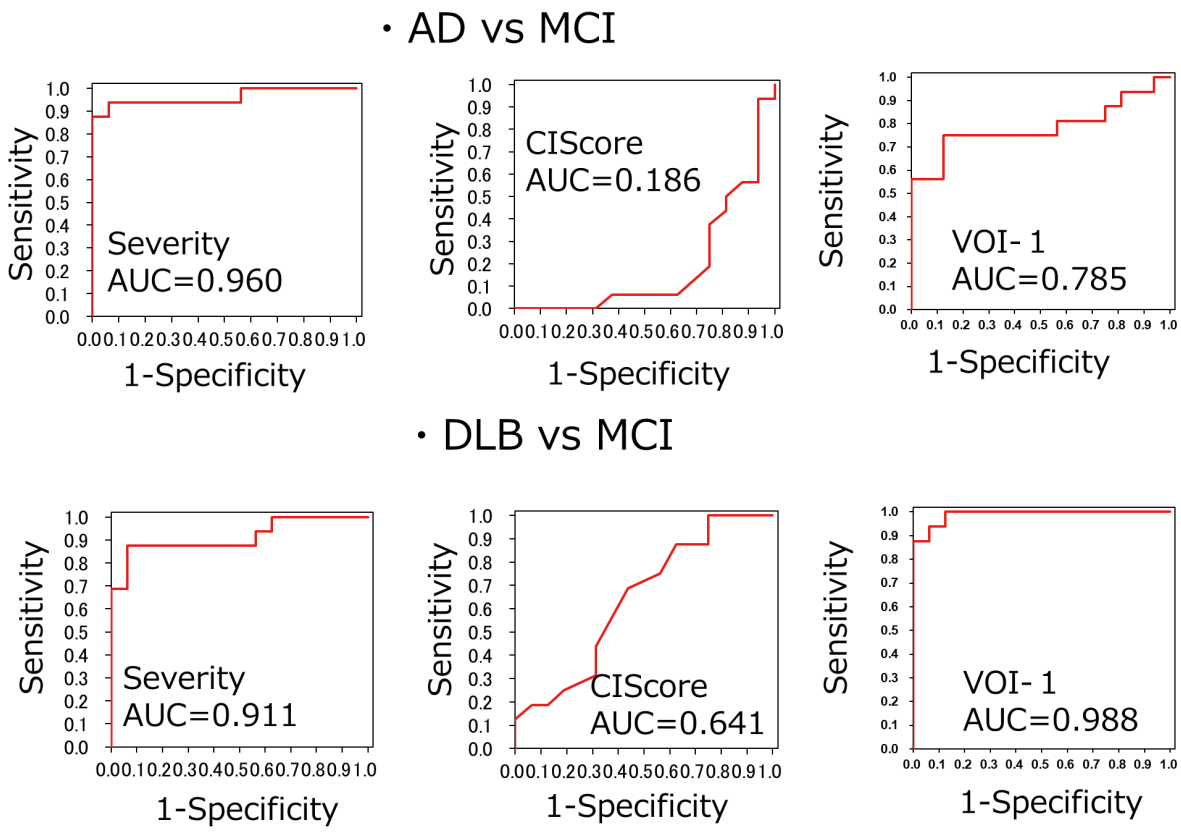
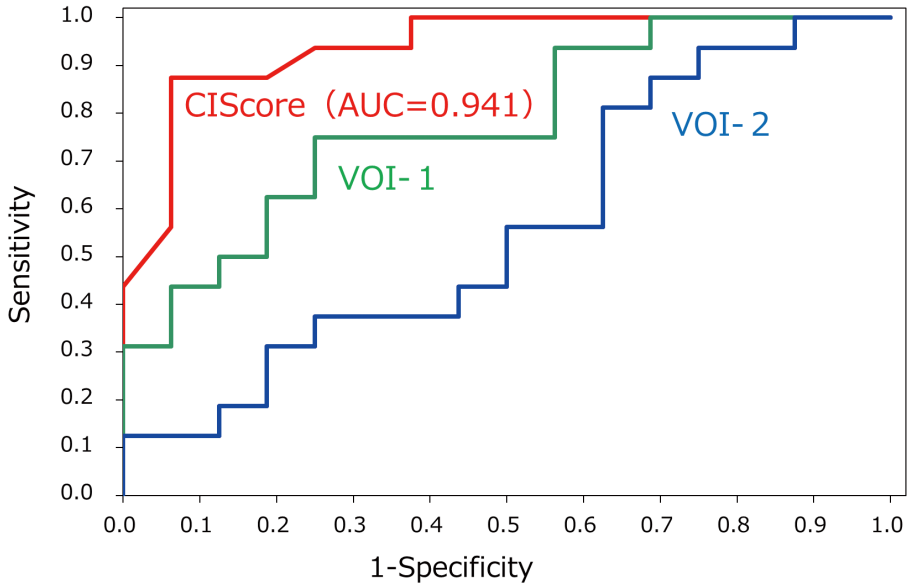


Figure 3 ROC analysis（AD vs MCI, DLB vs MCI）



set threshold of CIS in 0.225
Sensitivity : 87.5%、Specificity : 93.7%、Accuracy : 90.6%
PPV（Positive Predictive Value） : 93.3%
NPV（Negative Predictive Value） : 88.2%

Figure 4 ROC analysis（AD vs DLB）

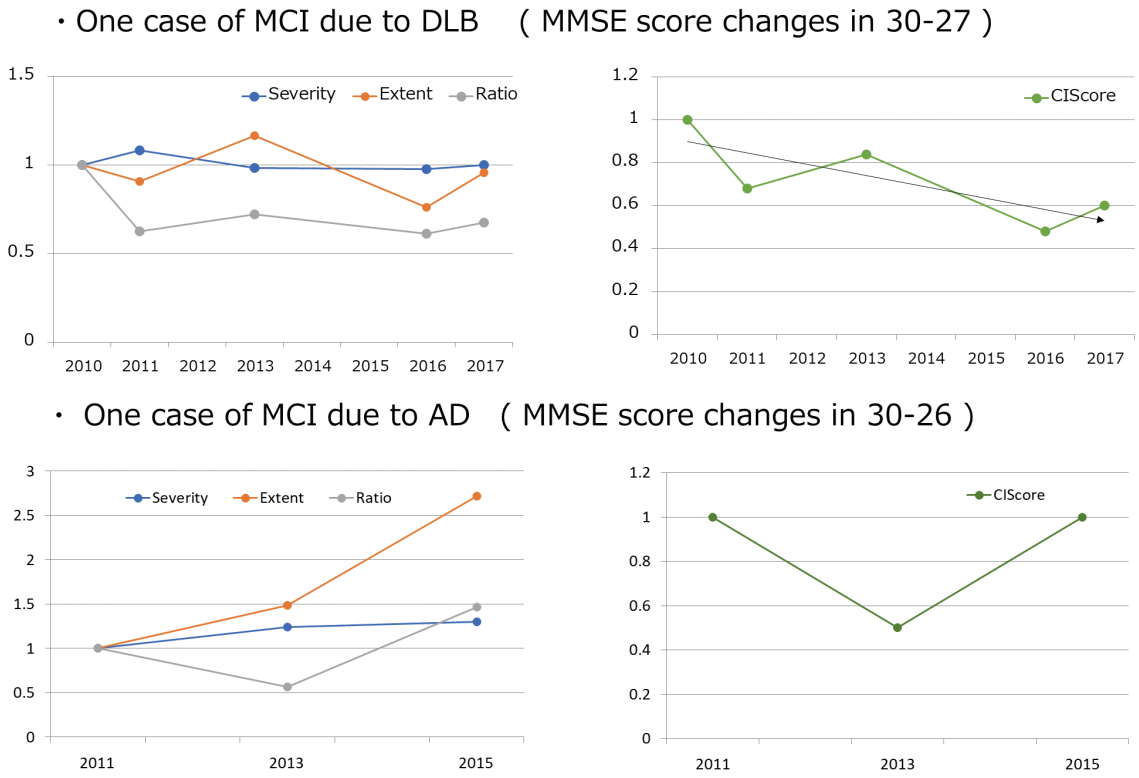


Figure 5 Rate of change from the testing first year (write an initial value for 1)

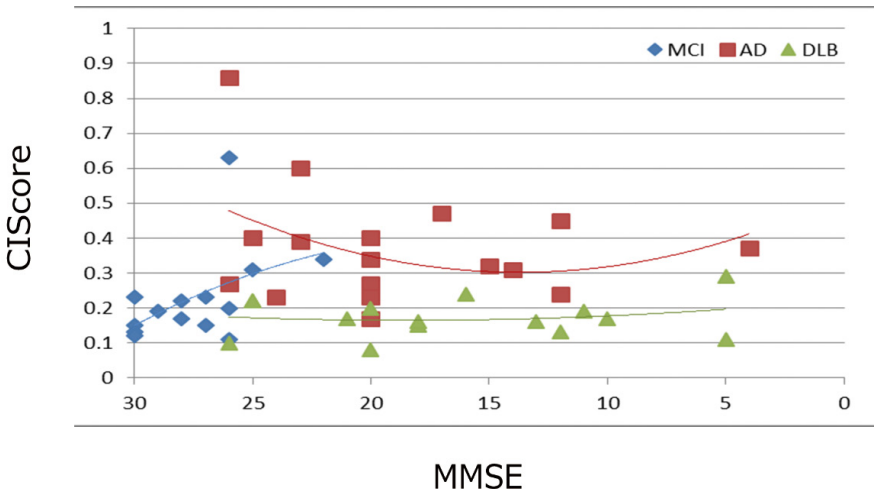


Figure 6 The correlation between MMSE and CIScore in each group

tial diagnosis. The severity and extent significantly increased in the AD and DLB groups. In other words, the significant increase in severity reflected the conversion and aggravation into AD and DLB. In contrast, the ratio was not significantly different between the groups, meaning that the ratio was almost constant regardless of the severity. These trends are consistent with previous studies using eZIS that involved healthy elderly people and mild to serious AD patients (Matsuda et al.)[14-16].

The above results suggest that severity and extent are useful indices for evaluating the conversion of MCI into AD and DLB. However, no significant difference was observed between the AD and DLB groups. Therefore, use of the VOI1, the volume of interest of DLB, is the most effective means of differentiating MCI from DLB.

In contrast, it was suggested that CIScore is not suitable for evaluating conversion from MCI to AD and DLB. The VOI1 is the volume of interest of

DLB, and the VOI2 is the area that remains after the VOI1 is subtracted from the SVA-A, the volume of interest of AD. Independently, these indices reflect the quantity of blood flow decrease in the respective areas. Significant difference was observed, suggesting the progression from MCI to AD or DLB. However, the CIScore, the ratio of these indices, did not show significant difference. The MCI group includes "due to AD" and "due to DLB", thus implying the possibility that characteristics of both diseases start developing from the MCI stage. Further research needs to be conducted to compare patients with healthy people and longitudinally evaluate indices. In the case of poor blood flow reduction, it is assumed that the above results were obtained because the relative difference increased by taking the ratio.

The CIScore was highly effective in differentiating early-onset AD and DLB. In a previous study, Imabayashi et al. used the CIScore for differentiating late-onset AD and DLB[13] (AUC0.882). The present study achieved a higher differentiating capability. This may reflect the fact that compared with late-onset dementia, blood flow decrease is more prominent in the VOI than in the area of brain atrophies in morphological imagery in early-onset dementia., and higher values tend to appear in the SVA[16]. Therefore, this suggests that it may be useful for differentiation in early-onset dementia. However, the fact that the clinical diagnosis also referred to the cerebral blood flow SPECT imaging findings may be the cause of the particularly high sensitivity and specificity levels.

In the MCI due to DLB group, the CIScore tended to decline over time in a single case. In other words, the CIS findings increased. Iizuka et al. have reported that the CIS findings increased as the MMSE score decreased down to around 22. However, below the score of 22, the CIS findings disappeared[17]. In the present study, where the final MMSE score was 27, the results were consistent with the tendency reported by them. Therefore, in the early-onset DLB group, the MMSE was predicted to correlate with the CIScore reflecting changes in blood flow. However, no apparent correlation was observed as a whole. Iizuka et al. have argued that the disappearance of the CIS findings is related to the progression of AD pathology, thus explaining the changes in the CIS findings by the chronological changes of the posterior cingulate cortex and precuneus plus cuneus[17]. In general, the neuropathological findings of DLB include numerous Lewy bodies in the cerebral cortex and AD pathological findings such as senile plaques and degraded neurofibrils (common form). In contrast, early-onset cases often exhibit only Lewy lesions without showing any AD lesions (pure form)[18]. Moreover, just like atrophies and tissue denaturation in the cerebral cortex differ between AD and SDAT, the characteristics of DLB were maintained in early-onset cases from MCI to more advanced conditions. Consequently, no correlation between the MMSE and CIScore was observed.

For differentiating early-onset dementia cases, eZIS was used. When severity was used for differentiating MCI from AD and DLB, the AUC values were 0.960 and 0.911, respectively. The CIScore was used for differentiating AD from DLB, and the threshold value was set at 0.225. The resultant AUC was 0.941, and the accuracy, sensitivity, and specificity were 90.6%, 87.5%, and 93.7%, respectively. The SVA-A was suggested to be useful for evaluating the conversion of MCI into both early-onset AD and DLB. Furthermore, the CIScore was suggested to be highly effective in differentiating AD from DLB not only in late-onset dementia but also early-onset dementia cases. It is important to understand the characteristics of each index and apply it to clinical practice.

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Author contributions

TO and HA were the doctors in charge of these cases and collected the data. MN analyzed and interpreted the data and wrote the manuscript. TT, KK, NS and YI provided guidance on manuscript writing. All authors have 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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Examination of a Low-density Lipoprotein Receptor Relative with 11 Ligand-binding Repeats (LR11) as a Biomarker in Esophageal Cancer

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Objectives: Some previous studies reported that the levels of a low-density lipoprotein receptor relative with 11 ligand-binding repeats (LR11) was a prognostic marker in some malignant tumors; however, whether LR11 is related to survival in patients with esophageal cancer remains unclear.

Methods: In this study, we measured LR11 in the preoperative serum of 46 patients of esophageal cancer who undergoing surgery using a sandwich enzyme-linked immunosorbent assay (ELISA) method with anti-LR11 monoclonal antibodies. We investigated the correlation between the level of LR11 and survival of patients with esophageal cancer. Clinicopathological data were retrospectively retrieved from our institution's database.

Results: The patients were divided into two groups (low LR11 and high LR11) based on the level of LR11. There was no statistical difference in clinicopathological factors between these two groups. The low LR11 group had a significantly longer overall survival than the high LR11 group.

Conclusions: LR11 can be measured with a relatively simple ELISA and is potentially a new prognostic marker for esophageal cancer.

Key words: esophageal cancer, lipoprotein receptor, LR11, biomarker

Introduction

Eleven ligand-binding repeats (LR11) is a receptor that shares a gene structure with the low-density lipoprotein (LDL) receptor and serves as an essential lipoprotein receptor that regulates the homeostasis of cholesterol metabolism. LDL receptors are thought to be involved in arteriosclerosis as well as in the migration of vascular smooth muscle cells[1]. It has been reported that the expression of the lipoprotein receptor related protein-1 or the LDL receptor-related protein 1B is an expression of a function-deficient gene family[2,3]. This gene family was identified from lung cancer and may be involved in the infiltration of tumor cells or metastasis[2,3]. LR11, which is part of the LDL receptor family, has been reported as a marker for Alzheimer's disease and arteriosclerosis[4], as well as for leukemia[5], and it has been reported that it may also be expressed in the serum of patients with cancer. However, there is no study for association between LR11 and esophageal cancer.

In this study, we examined the relationship between preoperatively collected serum LR11 levels in patients with thoracic esophageal cancer and survival to clarify whether LR11 is a useful prognostic factor for esophageal cancer.

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Methods

This study included a total of 46 patients diagnosed with esophageal cancer who underwent radical esophagectomy with three-field lymphadenectomy via a right thoracotomy and laparotomy at Juntendo University Hospital between September 2005 to December 2006[6]. Written informed consent was obtained from all enrolled patients. This study was conducted within the guidelines set by the Declaration of Helsinki and approved by the ethical committee of Juntendo Hospital (No. E21-0253). Blood was collected just before surgery. A total of 10 mL of collected blood was centrifuged, and the obtained serum was stored at -80℃.

The serum LR11 levels were measured by the sandwich enzyme-linked immunosorbent assay (ELISA) described below using anti-LR11 monoclonal antibodies. A total of 100 μL of eight-fold diluted serum was dispensed into three wells of an antibody-binding plate (anti-LR11 mouse monoclonal antibody, Sekisui Medical Co., Ltd., Tokyo) that was then left at room temperature (18-26℃) for 2 h. The liquid in the well was discarded, and the well was washed with 350 μL of cleaning solution (buffer solution, Sekisui Medical Co., Ltd., Tokyo). The washing solution was removed, and 100 μL of biotin-labeled antibody solution was added and left at room temperature for 1 h. The solution in the wells was then removed, and then the wells were washed. After washing, 100 μL of tetramethylbenzidine solution (Sekisui Medical Co., Ltd., Tokyo) was added and left to stand at room temperature, away from light to luminesce. Next, 100 μL of stop solution (sulfuric acid, Sekisui Medical Co., Ltd., Tokyo) was added to stop the reaction. A microplate reader was used to measure the absorbance at a wavelength of 450 nm.

The serum LR11 concentration measured by same method was reported around 10 ng/mL in most healthy individuals[7, 8]; thus, we divided the patients into a low LR11 group (LR11 levels below 10 ng/mL) and a high LR11 group (LR11 levels of 10 ng/mL or higher) and compared the two groups.

We examined the relationship between LR11 levels and clinicopathological findings of esophageal cancer. Additionally, we also examined its relationship with serum total protein (TP) level and albumin (Alb) level, which reflect nutritional status, and serum total triglyceride (TG) level and total cholesterol (T-Cho) level in serum. Treatment strategies were decided according to the Union for International Cancer Control (UICC) TNM classification 7th edition for esophageal cancer[9] in 2005 to 2006, so we analyzed based on this staging. Japanese Classification of Esophageal Cancer 11th edition was used for tumor location and vascular invasion evaluation[10, 11]. For statistical analyses, we used Kruskal- Wallis test and the Kaplan-Meier estimate for survival analysis (IBM SPSS Statistics ver.26).

Results

Patients' characteristics

Patients' characteristics are shown in Table 1. The serum LR11 levels ranged from 2.75 ng/mL to 21.4 ng/ mL. The average value across all patients was 6.86 ng/mL, and the standard deviation was 3.68 ng/mL (Figure 1).

After dividing the patients into the low-and high-LR11 level groups (cutoff value of LR11: 10 ng/mL), there were 39 patients in the low LR11 group and seven in the high LR11 group. There were no significant differences between the two groups in patients' characteristics.

Relationship between serum LR11 levels and pT staging

We examined the relationship between pT staging (classified as pT1, pT2, pT3, and pT4) and serum LR11 levels (Table 1). The serum LR11 levels tended to be lower in patients staged pT1, and to rise with increased tumor invasion degree; however, no significant differences were found (Figure 2).

Relationship between serum LR11 levels and pN staging

Regarding pN, the mean ± standard deviation values of serum LR11 in each group characterized as pN0, pN1, pN2 and pN3 showed no significant differences (Figure 3).

Relationship between serum LR11 levels and vascular invasion

We examined serum LR11 by classifying patients according to the degree of lymphatic invasion into ly0, ly1, ly2, and ly3 (Figure 4) and according to

Table 1 Clinicopathological background

		Low-level group (n)	High-level group (n)	
Gender	Male	34	5	NS
	Female	5	2	
Main tumor location*	Ce	0	1	NS
	Ut	7	0	
	Mt	19	3	
	Lt	11	3	
	Ae	2	0	
Histologic type				
Squamous cell carcinoma				
	Well	15	2	NS
	Mod	18	2	
	Poor	4	2	
Carcinosarcoma		1	1	
Malignant melanoma		1	0	
Depth of tumor invasion**	pT1	14	0	NS
	pT2	7	1	
	pT3	14	4	
	pT4	4	2	
Lymph node metastasis**	N0	12	3	NS
	N1	12	2	
	N2	11	0	
	N3	4	2	
pStage**	I	8	0	NS
	Ⅱ	11	4	
	Ⅲ	16	2	
	Ⅳ	4	1	

Ce: Cervical esophagus, Ut: Upper thoracic esophagus, Mt: Middle thoracic esophagus,
Lt: Lower thoracic esophagus, Ae: Abdominal esophagus, Well: Well differentiated squamous cell carcinoma,
Mod: moderately differentiated squamous cell carcinoma, Poor: poorly differentiated squamous cell carcinoma
*: Japan Esophageal, S; Japanese Classification of Esophageal Cancer, 11th Edition
**: UICC TNM Classification of Malignant Tumours, 7th ed
NS: Not Significant

degree of venous invasion into v0, v1, v2, and v3 (Figure 5). There were no significant differences between these groups.

Relationship between serum LR11 levels and blood biochemistry

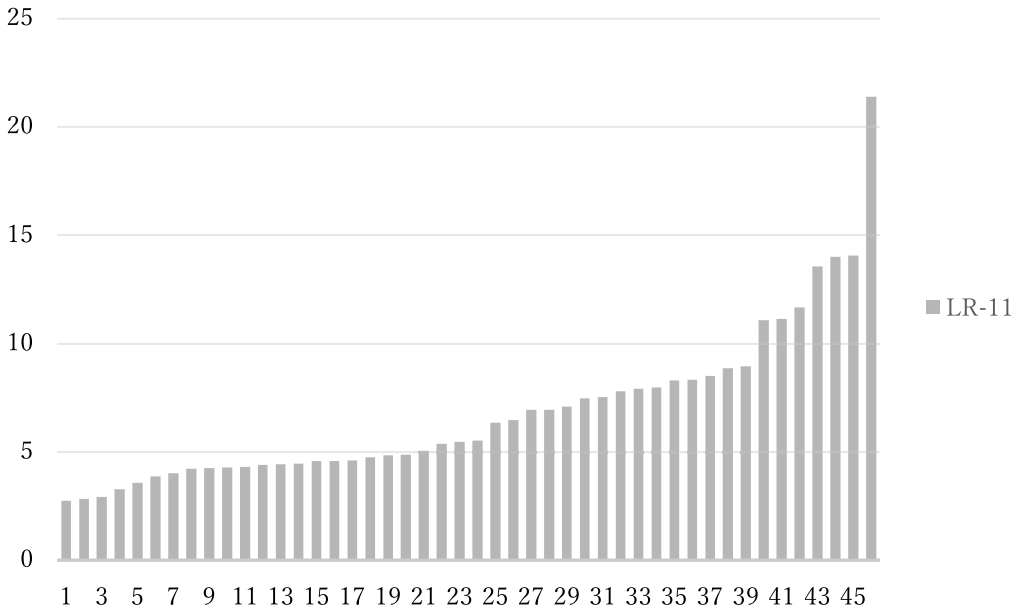
We examined the correlation between each of the blood biochemistry findings, as TP, Alb, TG, and T-Cho with serum LR11 levels; however, the results showed no significant differences for each value (Figure 6, 7).

Relationship between serum LR11 levels and survival

We compared overall survival of 39 patients in the low LR11 group and seven patients in the high LR11 group using the Kaplan-Meier estimate. Results showed that the 5-year survival rate was 41% in the low LR11 group and 14.3% in the high LR11 group, with a significantly worse prognosis for the high LR11 group (p=0.012, Figure 8).

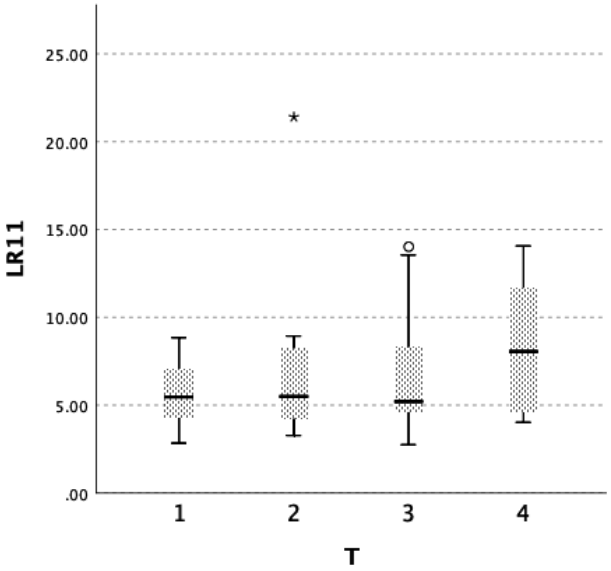
Discussion

LR11 was discovered by Jiang et al. in 2008 as an important factor involved in the differentiation of



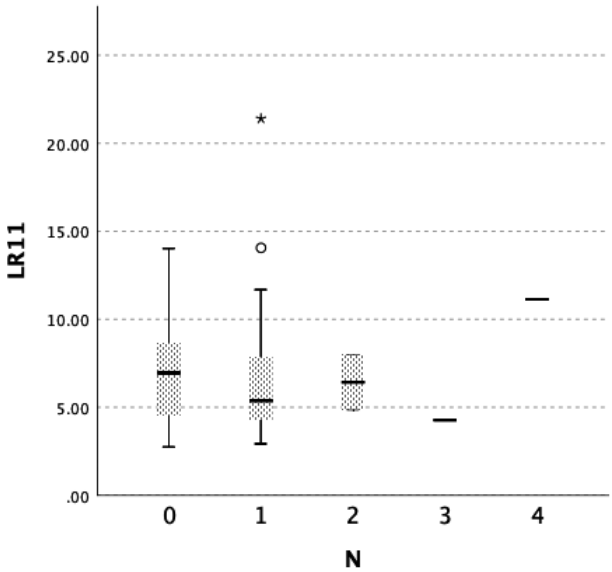
	Minimum value	Maximum value	Average value	Standard deviation
LR11(ng/ml)	2.75	21.4	6.86	3.68

Figure 1 Distribution of serum LR11 levels in patients with esophageal cancer



p=0.503

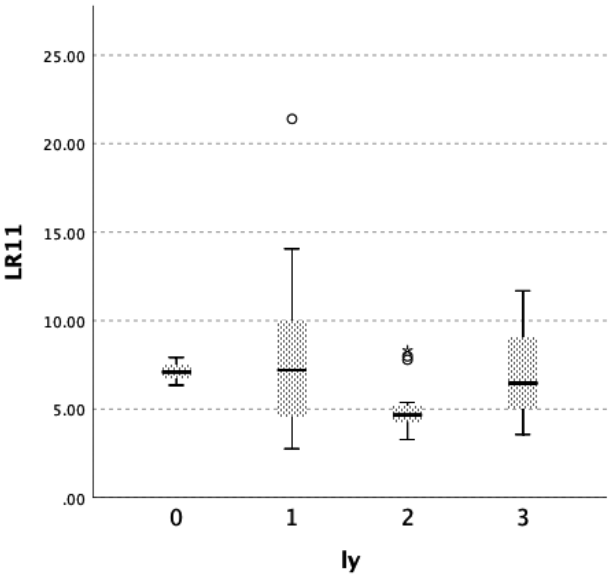
Figure 2 Depth of tumor invasion (T factor)* and serum LR11 levels in patients with esophageal cancer
T: UICC TNM Classification of Malignant Tumors, 7th edition



p=0.507

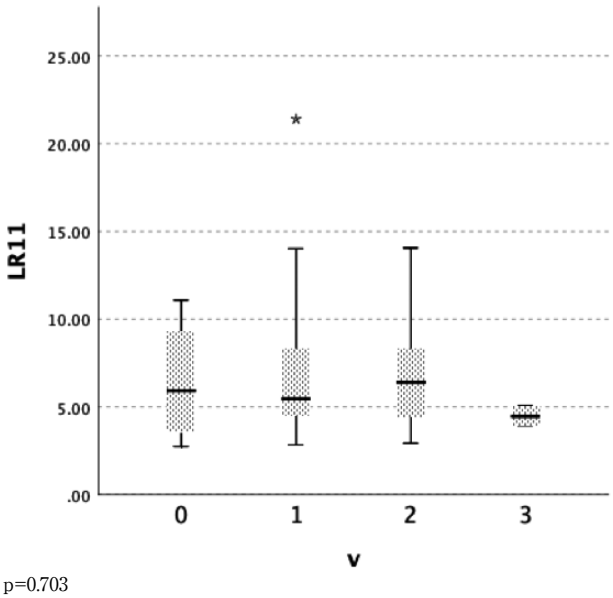
Figure 3 Lymph node metastasis (N factor)* and serum LR11 levels in patients with esophageal cancer
*: UICC TNM Classification of Malignant Tumors, 7th edition

undifferentiated vascular smooth muscle cells[12]. In 2010, Takahashi et al. found that serum soluble LR11 (sLR11) was an indicator of coronary artery stenosis[13]. Furthermore, it has been noted in recent years that sLR11 has characteristics as a biomarker for malignant tumors, where its levels increased with the exacerbation of acute leukemia, normalized with remission, and increased significantly with tumor infiltration into the bone marrow[14, 15]. Not only in the serum, LR-11 levels reported significantly higher in the bile from biliary tract cancer and pancreas cancer patients than in those



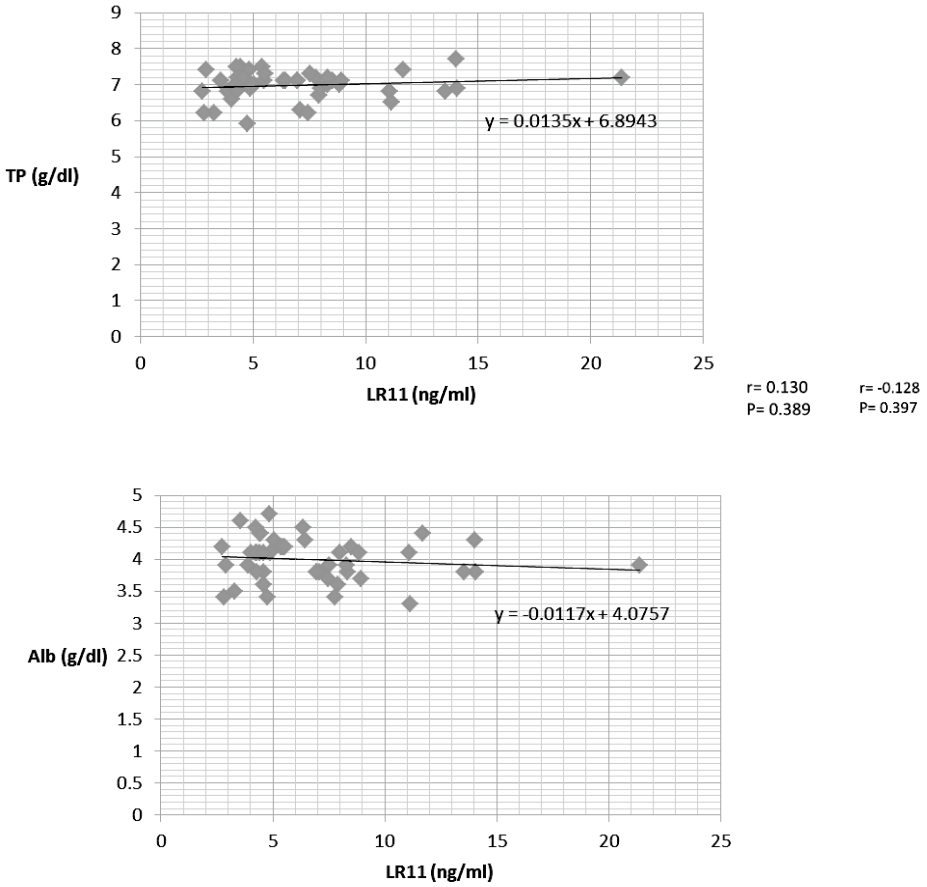
p=0.181
ly: lymphatic invasion

Figure 4 Lymphatic invasion (ly)[*2] and serum LR11 levels in patients with esophageal cancer
[*2]: Japanese Classification of Esophageal Cancer 11th ed



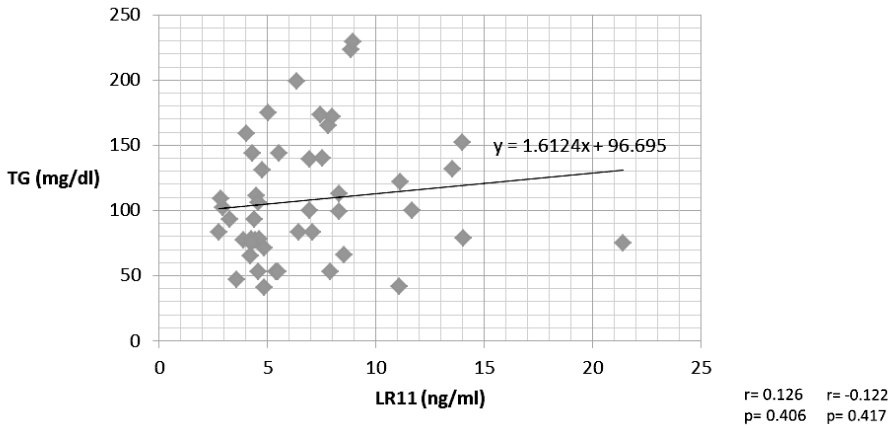
p=0.703
v: venous invasion

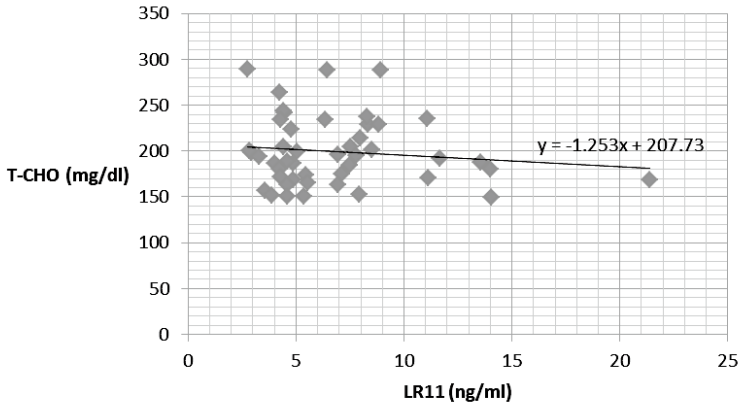
Figure 5 Venous invasion (v)[*2] and serum LR11 levels in patients with esophageal cancer
[*2]: Japanese Classification of Esophageal Cancer 11th ed



TP: Total Protein
Alb: Albmin

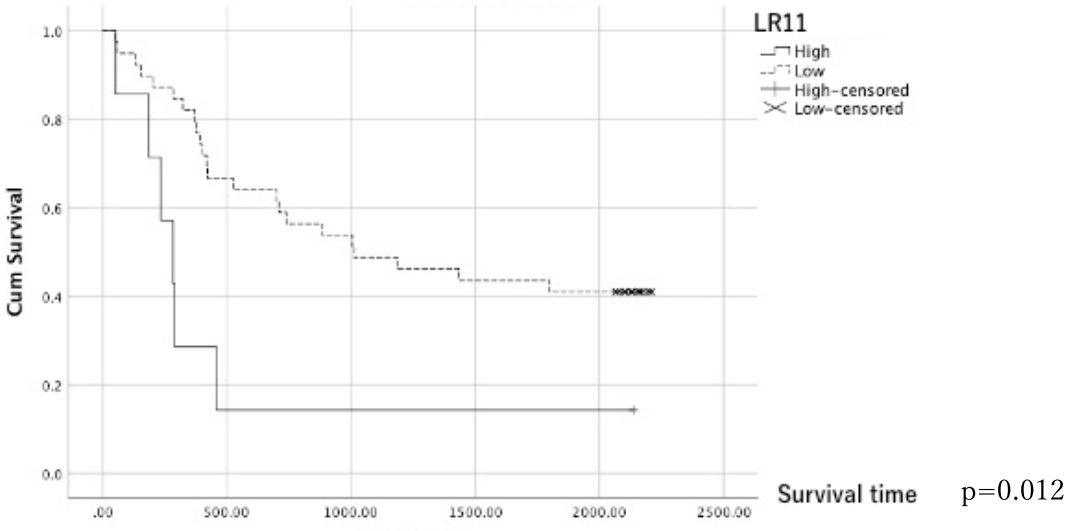
Figure 6 Total protein/albumin and LR11





TG: Total triglyceride
T-CHO: Total cholesterol

Figure 7 Total triglyceride / Total cholesterol and LR11



Median observation time:
Low level group:1012 days
High level group:283 days

Figure 8 Overall Survival

with benign diseases[16].

In this study, we hypothesized that serum LR11 level should rise in esophageal cancer patients, and examined the expression of LR11 in the serum of patients to evaluated the clinical significance of LR11. Thereby, the high serum LR11 level group had a significantly worse prognosis compared to the low LR11 group.

Firstly, the serum levels of patients with esophageal cancer were 6.86 ± 3.68 ng/mL; this value was relatively low when compared to values for patients with non-Hodgkin's lymphoma (17.7 ± 22.6 ng/mL), acute lymphocytic leukemia (73.5 ± 93.5 ng/mL), and acute myeloid leukemia (26.8 ± 29.1 ng/mL), where LR11 is already used as a biomarker[15–25]. It can be said that the value of serum LR11 in patients with esophageal cancer was lower than people without disease because the mean value of serum LR11 was reported to be around 10 as described above. We speculated that it was from patients' malnutrition because serum LR11 was related to metabolism of cholesterol and patients with esophageal cancer often become undernourished caused from esophageal stenosis. We compared LR11 levels with TP/ Alb, which reflects the nutritional status of the patient, and TG/ T-Cho, which reflects lipid metabolism in the patient; however, the results showed no significant differences. Therefore, we suspect that relatively low level of LR11 might be from differences between gastrointestinal solid cancers and hematological malignancies[26]. Recently, in patients with bile tract cancer and pancreatic cancer, bile sLR11 suggested to release from the cancer cell, and may reflect the characteristics of the microenvironment such as hypoxic conditions, and rapid cell proliferation[14–16]. Similarly, esophageal cancer might be affected by their microenvironment, so that it needs further study.

Based on our data, the level of serum LR11 are likely to be related to pT status, but not associated with pN, lymphovascular invasion, and other nutritional or lipid markers in blood. It is interesting that only pT status is likely to be related to serum LR11 because most prognostic biomarkers tend to be related to other significant prognostic markers like pT, pN, and lymphovascular invasions. We speculate that serum LR11 might not be related to the malignant potential but might be related to other factors such as size of tumors which could be influenced by food intake. In this study, we were not able to get information about how much weight patients lost before esophagectomy.

This study has some limitations. First, this study utilized a retrospective design. Second, the sample size was very small, and short period of observation. Moreover, the effect of another clinical features like adjuvant therapy, hyperlipidemia, and atherosclerosis need to be validated. Further, pathological evaluation of LR-11 expression in tumor tissue remains to be clarified, too.

In Conclusion, LR11 could be measured using the relatively simple ELISA method and is expected to be used as a new prognostic predictor for esophageal cancer.

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Author contributions

TU corrected blood samples, interpreted the patient data, and was a major contributor in writing the manuscript. The article was revised by MN and all authors read and approved the final manuscript.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

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Case Reports

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Postoperative Pyoderma Gangrenosum in a Laparoscopic Gastrectomy Port Site: A Case Report

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Background: Postoperative pyoderma gangrenosum (PPG) is a rare inflammatory skin disease of unknown etiology characterized by blistering and ulcerative lesions in postoperative wounds. Untreated pyoderma gangrenosum (PG) is potentially life-threatening; therefore, immediate and appropriate treatment is essential. Although PPG and surgical site infection (SSI) present similar clinical findings, they should be differentiated because of their conflicting treatment modalities.

Case presentation: An 82-year-old man with comorbidities of pulmonary tuberculosis, chronic obstructive pulmonary disease, and diabetes underwent laparoscopic gastrectomy for gastric cancer. On postoperative day 6, fever exceeding 39°C, port wound redness, and pain was observed. Laboratory tests revealed severe inflammatory reactions: white blood cell, 42,800/μL and C-reactive protein, 30.2 mg/mL. The patient was diagnosed with SSI and treatment with antibiotics and drainage was started; however, his general and wound conditions also worsened. Therefore, he was diagnosed with PG because painful skin findings were exacerbated by external stimuli and no significant bacteria were detected in the culture test. Treatment with oral prednisolone was started, which significantly improved his skin and inflammatory conditions.

Conclusion: We managed a rare case of PPG that occurred in a port wound after laparoscopic gastrectomy. If atypical clinical findings of postoperative SSI are observed, general surgeons should recognize and consider PPG as a differential diagnosis.

Key words: postoperative pyoderma gangrenosum, laparoscopic gastrectomy, surgical site infection

Background

Pyoderma gangrenosum (PG) is an uncommon, rare ulcerative skin disease characterized by a rapidly enlarging necrotic ulceration with an undermined border and surrounded by erythema[1,2)]. Untreated PG is potentially life-threatening; therefore, immediate and appropriate treatment is essential. Postoperative pyoderma gangrenosum (PPG) is also designed as postsurgical or pathergic PG and is closely associated with superficial granulomatous pyoderma[3)]. Although PPG and surgical site infection (SSI) presents similar clinical findings, they should be differentiated because of their conflicting treatment modalities.

Case presentation

An 82-year-old man with comorbidities of pulmonary tuberculosis, chronic obstructive pulmonary disease, and diabetes underwent upper gastrointestinal endoscopy performed by close examination of the black stool and was, therefore, diagnosed with

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gastric cancer. Laparoscopic distal gastrectomy was performed. The ports were positioned in a reverse trapezoid shape, as commonly practiced. The umbilical port was 12 mm in size. The port below the right hypochondrium was 5 mm in size, and the other three ports were 12 mm. The operation time and bleeding volume were 175 min and 35 ml, respectively, and no intraoperative complications occurred. Until postoperative day 5, only low-grade fever and prolonged inflammation were observed, but without specific clinical symptoms. On postoperative day 6, fever exceeding 39°C, port wound redness, and pain was observed. Laboratory tests revealed severe inflammatory reactions: white blood cell, 42,800/μL and C-reactive protein, 30.2 mg/mL. Computed tomography showed no postoperative intra-abdominal events but fluid retention in the subcutaneous tissue of the port site (Figure 1). He was diagnosed with SSI; therefore, the umbilical, right, and left latero-abdominal port wounds were opened and drained, and a Penrose drain® was placed. After administering broad-spectrum antibiotics and continuing the lavage treatment, the wound condition worsened, blisters and erosions were formed around it, and some necrotic findings with ulcers were also observed (Figure 2). Furthermore, the inflammatory reaction became even more severe in the laboratory test. The course did not match that of the typical postoperative SSI; therefore, we consulted with a dermatologist. The patient was diagnosed with PPG because of painful skin findings exacerbated by external stimuli and the absence of bacteria in the culture test. Skin biopsy also showed a high degree of neutrophil infiltration from the epidermis to the dermis and multilocular cysts, consistent with PG (Figure 3). The treatment with oral prednisolone of 50 mg/day was started, which significantly improved skin and inflammatory findings (Figure 4, Figure 5a, 5b). Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

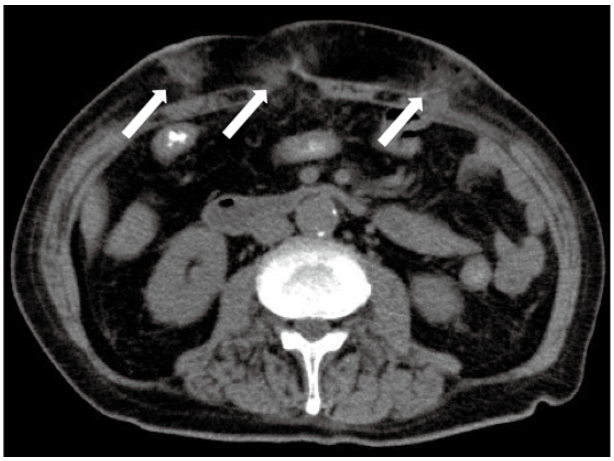

Figure 1 Computed tomography findings
No postoperative intra-abdominal events but fluid retention in the subcutaneous tissue of the port site.

Discussion

We managed a rare case of PPG occurring in a port wound after laparoscopic gastrectomy and required differentiation from SSI. Although the incidence of PPG is rare, most reports of PPG to date have come from dermatologists. Although very infrequent, it is one of the diseases that general surgeons should anticipate in differentiating atypical SSI.

PG is a neutrophilic dermatosis typically presenting as a small pustule, surrounded by a halo of inflammation that rapidly becomes painful ulceration with undermined wound edges and violaceous borders[4,5]. The cause of PG remains unknown, and its incidence is approximately 3-10 per million per

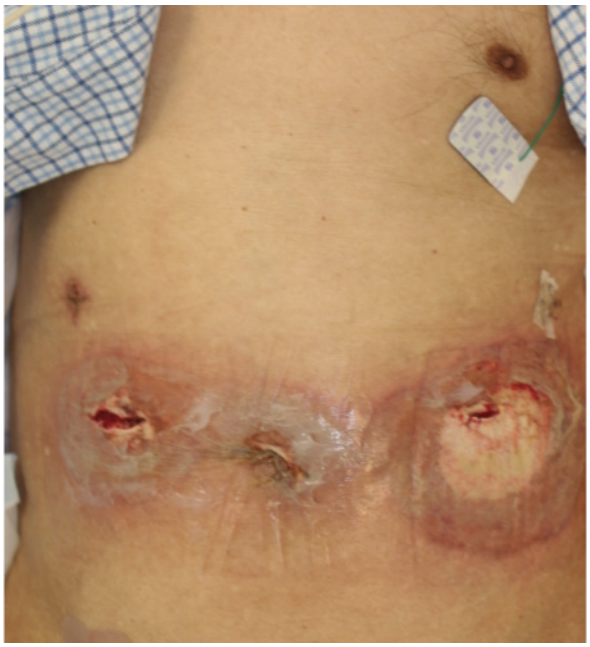

Figure 2 Skin findings on the sixth postoperative day after drainage treatment
Blisters and erosions were formed around it, and some necrotic findings with ulcers were also observed.

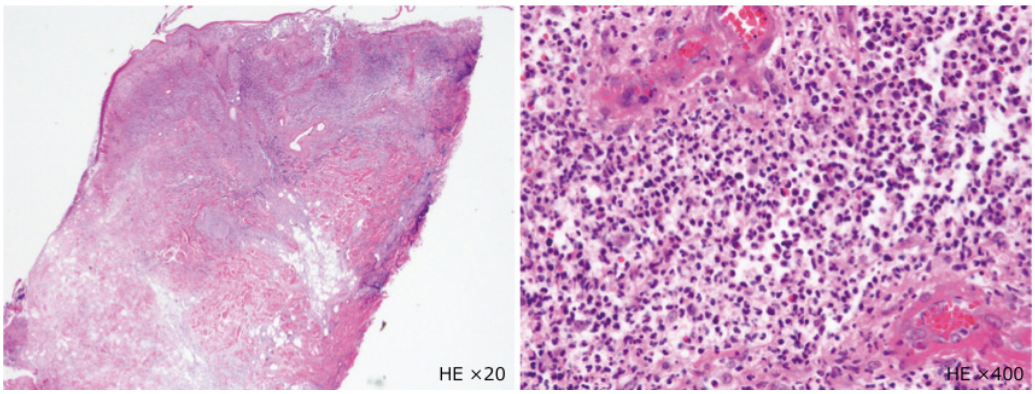

Figure 3 Pathological findings by skin biopsy
High degree of neutrophil infiltration from the epidermis to the dermis and multilocular cysts, consistent with PG.

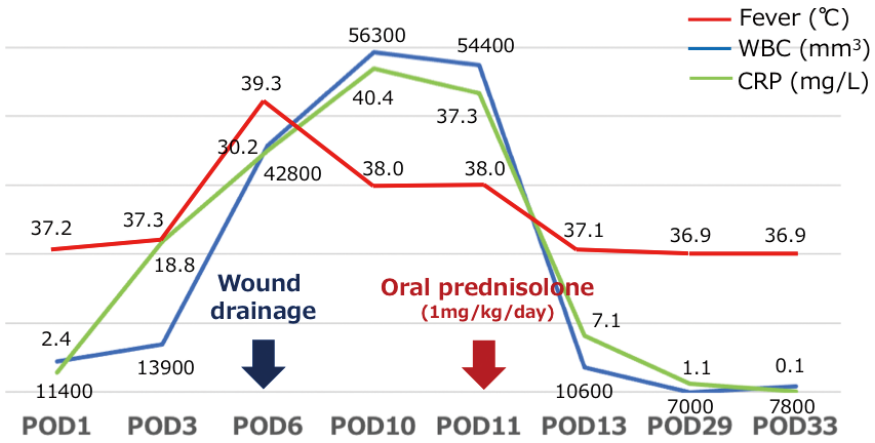

Figure 4 Changes in inflammatory findings
Inflammatory findings (fever, white blood cell, C reactive protein), which were aggravated by wound drainage, improved quickly after the start of treatment as PPG.

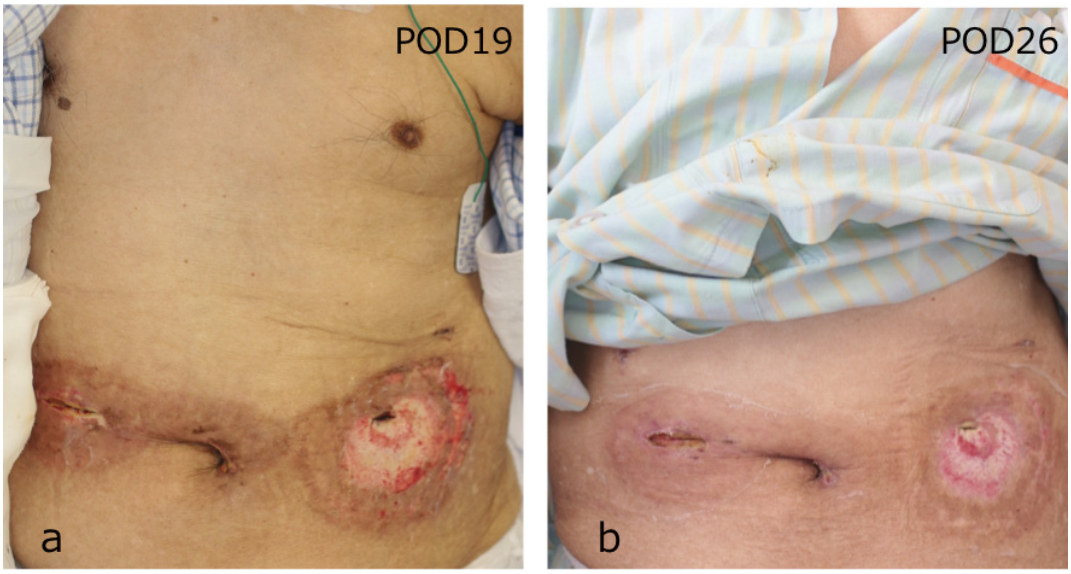

Figure 5 Changes in skin findings
In addition to oral prednisolone, wound treatment by maintaining a moist environment that avoids external stimuli resulted in significantly improvement.

year[6, 7]. PG is diagnosed by excluding other similar entities caused by infection, vasculopathies, neoplasms, and various inflammatory conditions[8]. Inflammatory bowel disease, rheumatologic disorders, and hematologic malignancies are comorbid conditions frequently associated PG[9]. Untreated PG is potentially life-threatening, and patients with PG have a three-fold increased risk of death compared to the general population; therefore, immediate and appropriate treatment is essential[10–12]. Aside from mortality, PG may cause pain and adversely impact the quality of life, predispose to secondary infection, disfiguring scarring, and recur[5, 7].

PPG is also classified as postsurgical or pathergic and is closely associated with superficial granulomatous pyoderma[3]. Systemic disease is reported to occur in 50%–78% of patients with PG, whereas a low prevalence of the systemic disease has been reported in PPG[6, 10, 13–15]. Suggesting that the coexistence of systemic diseases that are typical of PG may not be important in suspecting PPG. No comorbidity of systemic diseases related to PG was observed in this case. PPG commonly occurs in the breast, abdomen, and lower legs, in that order, at an average age of 50 years, and often occurs about 1 week postoperatively[15].

The common theme of all PPG literature is that the condition is almost always misdiagnosed as an SSI[15–18]. Stanislav et al. reported that 73% of patients with PPG were initially misdiagnosed as SSI and eventually treated[15]. Because fever, wound pain, and increased inflammatory response in blood tests and wound changes are also characteristic clinical findings of SSI; thus, the rarity of PPG can result in a false diagnosis. Currently, laparoscopic and robotic surgery have been introduced and widely performed in gastrectomy for gastric cancer due to their low invasiveness and cosmetic outcomes. The occurrence of SSIs is associated with an excess postoperative hospital stay, decreased quality of life, increased treatment costs, and increased mortality[19]. Although the standard of care for SSI is indisputably antibacterial therapy and appropriate debridement, a paradoxical relationship between SSI and PPG treatment must be considered. Our case was also regarded as SSI and treated with antibiotics, open drainage, Penrose drain® placed, and wound lavage; however, the wound condition wound severely deteriorated. One of the important points is that drainage, considered to be effective for wound infections, can worsen the wound condition in PPG[15, 20, 21]. Furthermore, no bacteria were detected in the wound culture test in PPG, and histological examination only shows nonspecific inflammatory findings, which will be useful in ruling out SSI. In the present case, only three of the five port wounds developed PG. The reason for this seems that all three port wounds were 12-mm ports, which are frequently manipulated intraoperatively, and the laparoscopy and forceps manipulations can easily cause strong physical irritation to the wounds, which is possibly contributed to the development of PG.

The treatment for PPG is generally the same as PG; corticosteroid and cyclosporine therapies are supported best by the literature[11, 22]. Zuo et al. reported that the majority of patients were treated with oral prednisolone (0.5–1.5 mg/kg/day) or intravenous methylprednisolone (0.5–1.0 mg/kg/day) combined with/without cyclosporine[18]. If the patient is resistant to these treatments, other treatments such as mycophenolate mofetil, infliximab, tacrolimus, or plasmapheresis may be considered[5, 21]. Moreover, steroid and immunosuppressive therapies for the treatment of PPG are predisposed to wound infections and can exacerbate existing infections; therefore, they should be used cautiously[5, 21, 23]. As for the treatment of the wound itself, as mentioned earlier, drainage should not be performed as it will worsen the condition. Maintaining a moist environment after washing with saline is important to promote proper wound healing[24]. Consistent with this case, systemic symptoms, laboratory findings, and wound condition dramatically improved with 50 mg (1 mg/kg/day) of oral prednisolone and wound care that avoided external stimuli as much as possible.

Although PPG is a rare disease, general surgeons should consider PPG as one of the differential diagnoses and treatments when managing an untypical course of SSI. Postoperative wound abnormalities that are resistant to the standard treatment should be referred to a dermatologist at an early stage of treatment, if possible, as the addition of expert judgment can lead to earlier diagnosis and treatment.

Conclusion

We managed a rare case of PPG that occurred in

a port wound after laparoscopic gastrectomy. If atypical clinical findings of postoperative SSI are observed, general surgeons should recognize and treat PPG as a possible differential diagnosis.

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Author contributions

SY, YA and KS drafted the manuscript. KS, YY and AK performed surgery. CJ, HE and YY contributed to patient care. TF, HO gave final approval of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest statement

The authors declare that they have no conflicts of interests.

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Publication List

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Publications from Juntendo University Graduate School of Medicine, 2020 [3/6]

Respiratory Medicine

〈Original Articles〉

＊ 1) Hayakawa D, Takahashi F, Mitsuishi Y, Tajima K, Hidayat M, Winardi W, Ihara H, Kanamori K, Matsumoto N, Asao T, Ko R, Shukuya T, Takamochi K, Hayashi T, Suehara Y, Takeda Nakamura I, Ueno T, Kohsaka S, Mano H, Takahashi K: Activation of insulin-like growth factor-1 receptor confers acquired resistance to osimertinib in non-small cell lung cancer with EGFR T790M mutation. Thorac Cancer, 2020; 11: 140–149.

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An asterisk (＊) denotes doctoral works by Japanese students.
A dagger (†) denotes doctoral works by non-Japanese students.

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編 集 後 記

当講座に基礎ゼミに来ていた学生が研究を行って論文発表をした。これに対して同窓会から奨励賞を出していただき、そして最近、その関連記事を当誌に掲載していただいた。大変結構なことである。

研究をある程度体験すると、科学的知見がどのような過程を経てもたらされるのか、研究手法の技術的限界の中で真実に辿り着くことがどのくらい困難で時として危ういものなのか、などを知ることができる。その結果、他の研究者から単純化されてもたらされる研究情報を、重層的に解釈できるようになる。これは、研究者だけでなく、臨床医にとっても重要なことであろう。この学生がどのような道を歩むかは分からないが、良い経験になったに違いない。

この基礎ゼミは医学部のカリキュラムの一部で、基礎医学の講義と実習を修めた後に行われ、研究者の視点で自然現象を見るよい機会となる。資格試験を通過することが中心となりがちな医学部教育にあって、大学としての高等教育を分かりやすく担保しているのが基礎ゼミであるのに、約1か月という申し訳程度の期間になっている。医学部以外のほとんどの学部では、学生に研究者としての活動を課していて、理系では研究室に、文系ではゼミに配属されて1－2年間の研究活動をしているようだ。医学部では医学専門教育が過密であり、その期間だけでは基礎ゼミが不十分である。ちなみに米国では、医学部進学の前に他の学部を卒業しているので、医学部入学時には研究活動を既に経験しており、部活動などで青春を謳歌することも堪能済みである。日本ではこれら医学専門教育、研究活動、その他の活動を大学生活に詰め込むことになるので、ほとんどの学生にとっては研究どころではない。

したがって、大学院に入って、4年間の研究活動をしてみる必要がある。4年間で一つの仕事をすると、専門外の研究を含めて、それなりに研究者目線が利くようになるはずだ。医学部と大学院はセットであると考えるべきだろう。

小西清貴
医学部生理学第一講座

イラスト作者より

せっかく奄美大島に行ったのに、あの美しい海と砂浜を描かないではいられず、前号に引き続きますが、描きました。海の色々なブルーはもちろんですが、波と風によって作られた砂の型と色（自然の造形美）に魅かれたのです。（宮道明子）

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(5), 564

順天堂大学40年間における泌尿器科診療の変遷

坂　本　善　郎

順天堂大学医学部附属練馬病院泌尿器科

私は1982年に順天堂大学泌尿器科学講座に入局し，2022年に定年退職するまで40年間順天堂で勤務いたしました．泌尿器科学の歩みは近年までゆっくりとしたものでしたが，この20～30年の間に急激な進歩を遂げました．順天堂での40年間を振り返り，この稿で簡単に記述してみます．特に，前立腺癌の診断と治療における進歩は目覚ましいもので，この点については，順天堂練馬病院での経験を中心に解説します．

キーワード：前立腺癌，経会陰的前立腺生検，ロボット支援前立腺全摘除術

この抄録は，順天堂醫事雑誌68巻5号，p454–458，2022掲載の『The Transition of Urological Practice for Forty Years in Juntendo University』の和文抄録です．

抄　録

順天堂醫事雑誌
2022. 68(5), 565

脳定位照射の歴史的変遷

直　居　　豊

順天堂大学附属練馬病院放射線科

順天堂病院はリニアックによる頭部定位照射をわが国で2番目に開始した病院です．開始当時から最近の定位照射の歴史的な変遷と治療技術の進歩，治療法の変化について私の勤務した順天堂医院と練馬病院を中心に報告する．順天堂医院がコバルトからリニアックに変わったのは1993年に新1号館がオープンした時で当時東芝製のリニアックが導入され，同時に白血病などに行う全身照射も開始いたしました．その1年後の1994年にリニアックによる頭部定位照射(いわゆるピンポイント照射)を開始しました．2005年には順天堂大学練馬病院が開院し，その年の9月から放射線治療が始まりました．当時の最新機種で順天堂関連病院では最初にIMRTと画像誘導放射線治療を開始した病院であります．2014年には本郷順天堂に2台目のリニアックが入り，本郷でもIMRT，画像誘導照射が可能となりました．そして最近の2021年には練馬病院で15年使用したリニアックが更新となり，最新式のリニアック治療装置が稼働し新しい方法で頭部の定位照射も開始しました．本稿では私が順天堂を中心に経験してきた頭部定位照射のその時々の手技と進歩について画像を提示しながら紹介します．

キーワード： 定位手術的照射，定位放射線治療，リニアック，ガンマナイフ

この抄録は，順天堂醫事雑誌68巻5号，p459–464，2022掲載の『Historical Review of Stereotactic Radiosurgery in Juntendo University』の和文抄録です．

抄　録

順天堂醫事雑誌
2022. 68(5), 566

With/afterコロナ時代の新たな感染対策：二酸化塩素は有効かつ安全か？

大 日 方　薫

順天堂大学医学部集団感染予防学講座

最近の新型コロナウイルス(SARS-CoV-2)感染症(COVID-19)の世界的な大流行から有効で安全な感染予防対策が求められるようになった．気道感染症ウイルスは主にエアロゾルを介して速やかに伝播するためクラスターや集団感染を引き起こす．室内のエアロゾル感染対策としてオゾン，二酸化塩素ガスが有用とされている．しかし，オゾンは比較的高濃度が除菌に必要となるため，人体に対する毒性が問題となる．一方，低濃度の二酸化塩素は有人環境下であっても有効かつ安全な感染対策に用いることが可能である．さらにHEPAフィルターの併用により，より効果的で安全な感染対策になることが期待される．

キーワード：二酸化塩素，COVID-19，エアロゾル感染，除菌

この抄録は，順天堂醫事雑誌68巻5号，p465-472，2022掲載の『New Countermeasures Against Infections with/after COVID-19: Is Chlorine Dioxide a Useful and Safe Disinfectant?』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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