Table 1
Patients characteristics.

Case	Age	Site	Primary/lymph	Rec/fresh	TNM	Size (cm)	Histology	ICG score
1	57	Hypopharyx	Lymph node	Recurrence	T2N2bM0	2.3	SCC	5
2	37	Parapharyngeal space	Primary	Fresh	T2N2bM0	2.5	MP	5
3	82	Oropharynx	Lymph node	Fresh	T4N2cM0	2.5	SCC	4
4	78	Maxillary	Lymph node	Recurrence	T4N2bM0	2.3	AC	4
5	52	Hypopharyx	Lymph node	Fresh	T2N2bM0	2.2	SCC	4
6	60	Oropharynx	Lymph node	Fresh	T2N2bM0	3.7	SCC	4

SCC: squamous cell carcinoma, MP: malignant paraganglioma, AC: adenocarcinoma, rec: recurrence, size: parapharyngeal tumours.

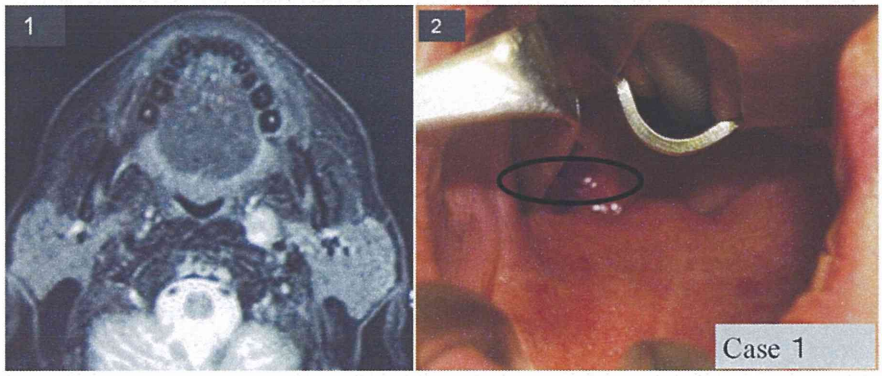

Fig. 1. Case 1 Hypopharyngeal cancer (squamous cell carcinoma). Recurrent retropharyngeal metastasis was resected transorally, guiding ICG imaging. 1: MRI. 2: Tumour location was marked based on ICG image (circle).

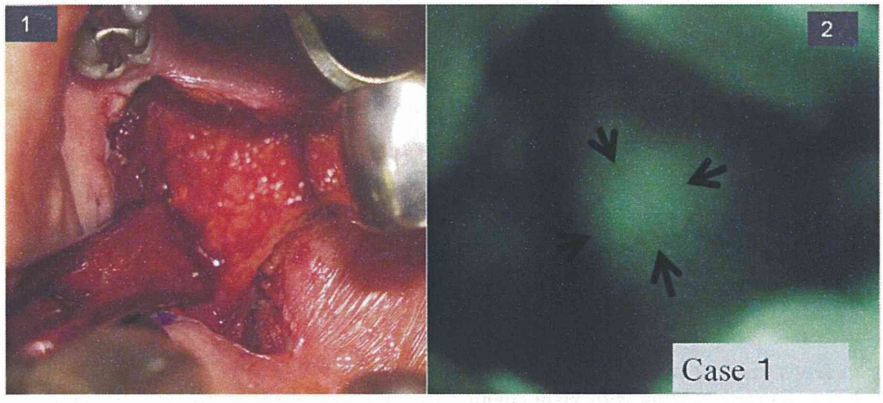

Fig. 2. Case 1 Operative findings. 1: Submucosal tumour was visually obscured by fasciae. 2: ICG image indicated the obscured tumour (arrows). The tumour was removed while preserving function.

avoid stretching the important organs and prevent damage to the carotid artery and lower cranial nerves. As a result, we were able to successfully separate the tumours from the carotid artery and lower cranial nerves while protecting organs.

This protocol was approved by Juntendo University IRB.

3. Results

All six tumours displayed bright fluorescence emissions which clearly contrasted with the normal structures. Even with the submucosal tumour covered with and obscured by fascia, we were able to observe the tumour clearly under HEMS imaging. NIR Fluorescence Imaging Visibility Score (Poellinger et al., 2011) was not different between squamous cell carcinoma and other malignant tumours.

The tumour was completely resected pathologically with its capsule. As a result, we were able to remove the tumour safely and noninvasively to preserve pharyngeal functions. These findings are particularly useful for detection and safe resection of tumours invading the parapharyngeal space. We show typical cases of parapharyngeal space.

Parapharyngeal tumours were resected by a transparotid procedure. The tumours were located behind the carotid sheath in the parapharyngeal space, making it difficult to physically palpate them or to observe the invaded deep organs. In order to visualize and safely resect these tumours we employed ICG for navigation surgery. ICG florescence imaging with HEMS revealed the tumour behind carotid sheath (Fig. 3). Consequently, all parapharyngeal tumours were completely and safely resected by ICG navigation surgery without complications.

4. Discussion

Optical imaging using ICG is a new technique for visualizing tumours during surgery. Some reports have indicated that ICG NIR fluorescence imaging guided surgery is efficient in liver cancer

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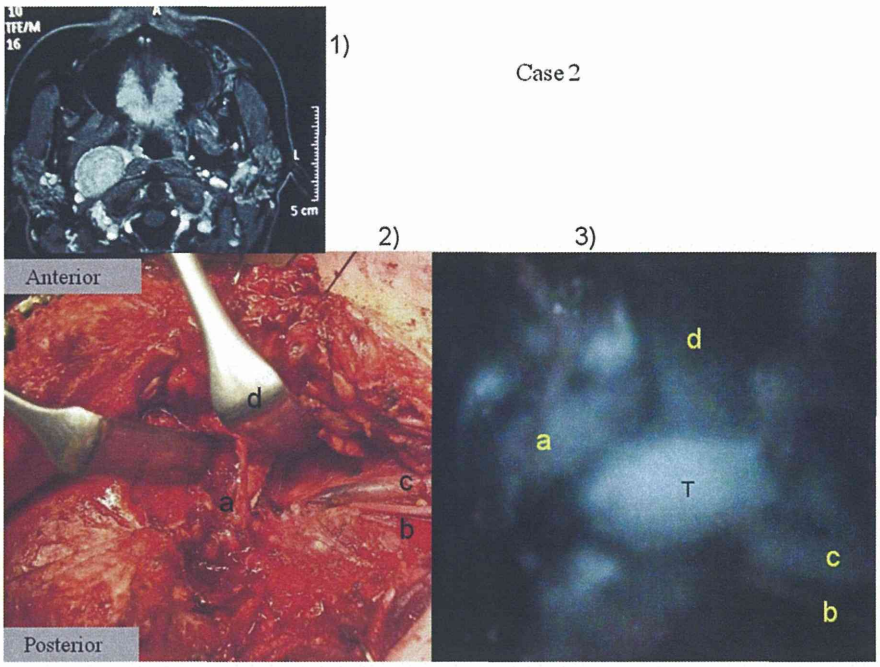

Fig. 3. Case 2 Parapharyngeal space tumour (malignant paraganglioma). 1: MRI. 2: Intraoperative findings a) facial nerve, b) accessory nerve, c) internal jugular vein, d) retractor. 3: ICG navigation surgery for parapharyngeal space tumour a) facial nerve, b) accessory nerve, c) internal jugular vein, d) retractor.

surgery to detect not only large cancers, but also when detecting micro cancers (Ishizawa et al., 2009). In this study we suggest the usefulness of ICG fluorescence imaging not only in liver cancer surgery, but also in head and neck cancer surgery (Yokoyama et al., 2011; Fujimaki et al., 2012).

We also used a new HEMS handheld device for the first time. This is a high sensitivity charge-coupled device camera and custom made optical filter with non-Bayer colour filter arrays. It can detect visible and near-infrared (NIR) rays from 380 to 1200 nm without bias in colour balance at 30 frames per second (Handa et al., 2010). It has high sensitivity due to the colour CCD camera used in ICG fluorescence imaging (Yokoyama et al., 2011).

The handheld HEMS can detect pharyngeal tumours behind oral cavity or nasal cavity by ICG-enhanced imaging with vivid colour and can be freely operated intraoperatively by surgeons. We can find the ICG Fluorescent Image in necrotic tumour tissue. In almost all cases, necrotic tumour tissue was located in central zone in metastatic lymph nodes and we can find ICG Fluorescent Image around surface viable tumours.

Because of the high sensitivity of ICG fluorescence imaging, ICG fluorescence imaging under HEMS can help to resect tumours which have invaded the parapharyngeal space behind the internal carotid artery, internal jugular vein, and lower cranial nerves. Macroscopic imaging with HEMS can allow adequate tumour-free margins while minimizing surgical morbidity and preservation of function (Yokoyama et al., 2011). In addition, we can confirm ICG Fluorescent Image tumour region pathologically.

We also have demonstrated a successful method of distinguishing cancer from normal tissue and optimum surgical time with HEMS in animal models (Fujimaki et al., 2012). Application of endoscopic and robotic surgery for the parapharyngeal space lesions enables minimally invasive surgery with superior results (Desai et al., 2008). However, we need to be able to detect parapharyngeal tumours in deeper and in invisible areas when palpation is not possible. This is required in order to resect tumours safely through tumour detection carried out with ICG fluorescent imaging.

Because of the high sensitivity, HEMS imaging facilitates safe resection of parapharyngeal tumours. Further investigations may lead to the development of a new minimally invasive surgical therapy achieving both better prognosis and function preservation in head and neck cancer.

5. Conclusion

ICG fluorescence imaging is effective for the detection and resection of the parapharyngeal space tumours.

Authors' contributions

JY conceived of the study design. JY prepared and edited the manuscript. SO and MF contribute to acquisition of data. TA, RY, and MK performed the statistical analysis. JY and KI gave the final approval of the version of the manuscript. All authors read and approved final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Original Research

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Novel Indocyanine Green–Phytate Colloid Technique for Sentinel Node Detection in Head and Neck: Mouse Study

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Abstract

Objective. Sentinel node navigation surgery using real-time, near-infrared imaging with indocyanine green is becoming popular by allowing head and neck surgeons to avoid unnecessary neck dissection. The major drawback of this method is its quick migration through the lymphatics, limiting the diagnostic time window and undesirable detection of downstream nodes. We resolved this problem by mixing indocyanine green (ICG) with phytate colloid to retard its migration and demonstrated its feasibility in a nude mouse study.

Study Design. Experimental prospective animal study.

Settings. Animal laboratory.

Subjects and Methods. Indocyanine green at 3 concentrations was tested to determine the optimal concentration for sentinel lymph node detection in a mouse model. Effect of indocyanine green with phytate colloid mixture solutions was also analyzed. Indocyanine green or mixture solution at different mixing ratios were injected into the tongue of nude mice and near-infrared fluorescence images were captured sequentially for up to 48 hours. The brightness of fluorescence in the sentinel lymph node and lymph nodes further downstream were assessed.

Results. Indocyanine green concentration >50 μg/mL did not improve sentinel lymph node detection. The addition of phytate colloid to indocyanine green extended the period when sentinel lymph node was detectable. Second echelon lymph nodes were not imaged in mice injected with the mixture, while these were visualized in mice injected with indocyanine green alone.

Conclusion. This novel technique of ICG–phytate colloid mixture allows prolonged diagnostic time window, prevention of downstream subsequent nodes detection, and improved accuracy for the detection of true sentinel lymph nodes.

Keywords

sentinel node navigation surgery, head and neck cancer, indocyanine green, phytate colloid

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Introduction

Most cancers of the head and neck are squamous cell carcinomas (HNSCC) that arise from the mucosal surfaces of the upper aerodigestive tract. The standard treatment options for HNSCC are surgery and/or radiotherapy, with or without chemotherapy. However, aggressive treatment of HNSCC by these modalities often results in functional disorders such as loss of voice or severe dysphagia. Therefore, there is a need for less invasive treatment for HNSCC that would improve prognosis while maintaining a good quality of life.

HNSCC are known to have high rates of lymphatic metastasis even in early T stages.[1] The control of lymphatic metastasis is one of the most important issues in the treatment of HNSCC, since it has been proved to be the most important prognostic factor in these malignancies. Even occult metastases can become overt if not treated properly, but current imaging tests such as computed tomography

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(CT), magnetic resonance imaging (MRI), ultrasonography (US), or positron emission tomography (PET) are not reliable enough for detecting occult lymph node (LN) metastases of the neck in patients clinically staged as N0.[2] Therefore, the decision between elective neck dissection and watchful waiting in patients without clinically apparent lymph node involvement is controversial at present.[3,4]

Sentinel lymph node navigation surgery (SNNS) can allow head and neck surgeons to avoid unnecessary neck dissection and reduce the incidence of postoperative morbidities such as facial edema, limited range of motion of the upper limbs, and shoulder pain. Several researchers have reported the successful application of SNNS for the management of HNSCC patients.[5-8] Since minimally invasive, organ-preserving surgery for the primary lesions of HNSCC has become popular with the development of trans-oral resection procedures such as trans-oral laser microsurgery (TLM),[9] trans-oral robotic surgery (TORS),[10] or trans-oral video-laryngoscopic surgery (TOVS),[11-14] the combination of these trans-oral surgical procedures with SNNS can provide minimally invasive treatment options with preservation of both organ and function for patients with both primary lesions, as well as those with lymphatic metastasis lesion.

Radionuclide (RN) methods using technetium-99m (^{99m}Tc)-radiocolloid are the best for accurately identifying sentinel lymph nodes (SLNs). However, RN methods have drawbacks, including radiation exposure and the consequent need to conform to statutory regulations that complicate the performance of SNSS for HNSCC. The high cost of a gamma probe is another impediment to the applicability of RN methods in SLN detection during surgery. In addition, the shine-through phenomenon encountered during the use of RN compounds generally hampers SLN detection, especially for HNSCC where the SLN is often located near the tracer injection site.

Near-infrared fluorescence (NIR) imaging has the potential to address the drawbacks of RN methods by allowing real-time optical detection of the SLN in the midst of surrounding tissues.[15-18] SNNS techniques using real-time indocyanine green (ICG) fluorescent imaging are widely used in breast and gastric cancer.[19,20] One of the advantages of these methods is that these can be easily used in the operation room. These enable technically easy and accurate tracer injection, compared to presurgical RN injection techniques into the larynx or hypopharynx under flexible endoscope guidance. Even though some reports have demonstrated improved detection sensitivity with the ICG method,[21] it has a few problems, especially in the head and neck region. SLNs located in deeper tissue are difficult to detect because signal penetration of fluorescent probes is limited by tissue attenuation.[22] Compression of the neck from the outside is one way to resolve this problem.

Another major problem of ICG is its quick migration through lymphatic channels, making it difficult to detect the true SLN. The problem is compounded by the undesirable visualization of other lymph nodes lying downstream.[23] Together, these can lead to serious failure in detecting the true SLN with the help of ICG while performing SNNS in the head and neck region. A novel attempt was made to overcome this disadvantage by modifying the ICG method with the addition of phytate colloid. The feasibility of this method was assessed in a nude mouse study. Phytate colloid has been commercially available for a long time in Japan as a low-price instant-labeling hepatic scanning agent. As phytate colloid can easily form small-sized colloid particles in solutions such as serum that contain calcium ions, it is suitable for SLN detection. The use of this compound for SLN mapping has recently been covered by public health insurance in Japan.

The objective of this study was to assess the SLN detection ability of our novel modified ICG technique in which ICG is mixed with phytate colloid in a mouse model. The optimal ICG concentration for SLN detection in the mouse model was first determined. Subsequently, ICG at the optimal concentration was mixed with phytate colloid to evaluate if the mixture could overcome the problem of quick migration of ICG in the conventional ICG method.

Materials and Methods

Animals

The use of animals in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the National Defense Medical College. Animal experiments were conducted using 5- to 8-week-old athymic BALB/c nu/nu nude mice weighing 20 to 25 grams. All animal procedures were performed under general anesthesia by the intraperitoneal (ip) injection of medetomidine (0.8 mg/kg, ip) and ketamine hydrochloride (40 mg/kg, ip), or inhalation anesthesia with isoflurane. The depth of anesthesia was determined by toe pinch.

Preparation of ICG and ICG–Phytate Colloid Mixture

To prepare the stock solution, 25 mg of ICG (Diagnogreen for injection, Daiichi-Sankyo Co, Ltd, Tokyo, Japan) was dissolved in 5-mL sterile water. The stock solution was diluted with sterile water to make working solutions of final concentrations of 5000 μg/mL (6400 μM, stock solution), 500 μg/mL (640 μM, 10 times dilution), and 50 μg/mL (64 μM, 100 times dilution).

Phytate colloid solution was prepared according to the manufacturer's instruction manual of the Techne phytate kit (FUJIFILM RI Pharma Co, Ltd, Tokyo, Japan). ICG–phytate colloid mixture was prepared by mixing ICG with phytate colloid solution with different mixing volume ratios of 1:0 (ICG alone), 1:9, or 1:99. Final concentration of ICG was adjusted to 50 μg/mL (64 μM) in all mixtures.

Effect of ICG Concentration for SLN Detection

The effect of ICG concentration was first evaluated to find out its optimum concentration in the mouse model. Twelve nude mice were assigned to 3 groups of 4 mice each. Ten μl of ICG of the following concentrations (5000, 500, and 50 μg/mL) was injected with a 29 gauge needle into the tongue of nude mice of each group, respectively. SLN was detected as a fluorescent spot shining through the skin under a near-infrared

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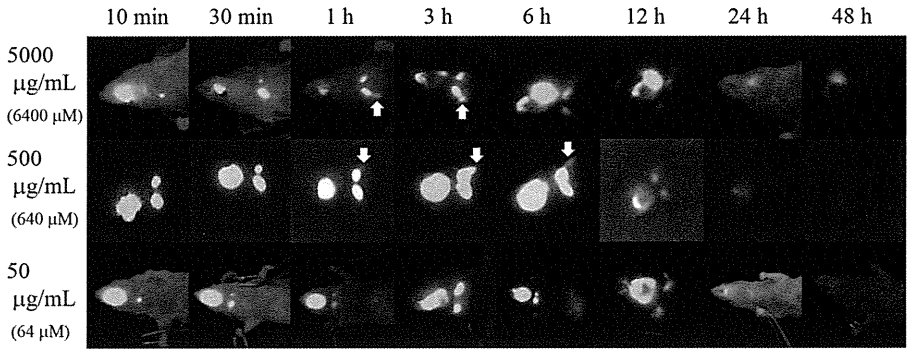

Figure 1. Effect of different indocyanine green (ICG) concentrations. Sentinel lymph nodes (SLN) fluorescence was immediately visible, peaked at 3 to 6 hours, and generally disappeared by 24 hours. Second echelon LNs often appeared after 30 to 60 minutes. White arrow: second echelon LN.

fluorescence imaging system (Photodynamic eye, Hamamatsu Photonics K.K., Hamamatsu, Japan). Fluorescent images were photographed sequentially at 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours after ICG injection. The first fluorescent spot was defined as the SLN; fluorescent spots detected afterwards on the same side were defined as second echelon lymph nodes. The relative brightness of the SLN was determined by measuring the signal-to-background ratio (SBR).[24]

Effect of ICG–Phytate Colloid Mixture on SLN Detection

Twelve mice were assigned to 3 groups of 4 mice each to evaluate the effect of different mixing ratios of ICG–phytate colloid on SLN detection. Ten μl of the ICG–phytate colloid mixture solution of different mixing ratios (ICG alone, 1:9 or 1 part of ICG in 9 parts of phytate colloid solution, and 1:99 or 1 part of ICG in 99 parts of phytate colloid solution) was injected into the tongue of the mice with a 29 gauge needle. ICG concentration was 50 μg/mL in all solutions (settled by the results of previous experiment).

SLN and second echelon LNs were detected with a near-infrared fluorescence imaging system the same way as in the experiment on determining optimal ICG concentration. Fluorescent images were captured sequentially 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours after ICG–phytate colloid mixture solution injection. Relative brightness of SLN was determined by measuring SBR.

Statistical Analysis

Statistical analysis was performed with the nonparametric Mann-Whitney U test. P values $< .05$ were considered statistically significant. All values were expressed as the mean ± standard error.

Results

Effect of ICG Concentration for SLN Detection

Figure 1 shows the results after the injection of different concentrations of ICG. SLN was visible just after injection

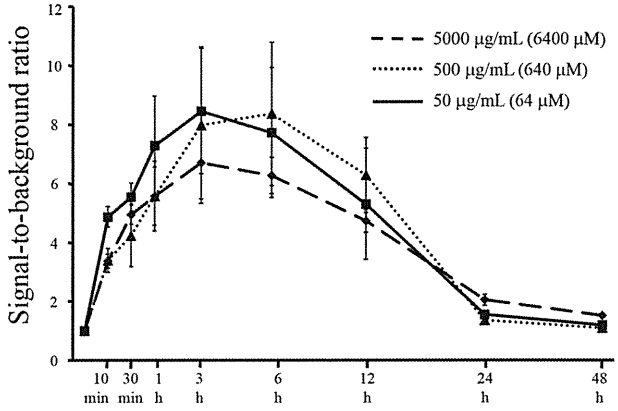

Figure 2. Effect of different indocyanine green (ICG) concentrations on sentinel lymph nodes (SLN) fluorescence brightness. No significant difference was observed in SLN fluorescence brightness among the groups injected with different concentrations of ICG.

in all groups. The fluorescent intensity of SLN reached its maximum level around 3 to 6 hours after injection, then slowly decreased, and practically disappeared 24 hours after injection in all groups. Second echelon LNs were detected after 30 to 60 minutes in many mice. No obvious toxicity was found in all mice.

Figure 2 shows the change in SBRs measured over time. Signal intensity increased right after injection, peaked after around 3 to 6 hours, and decreased in all groups. No significant difference was observed in the trend of change in fluorescent intensity among the groups. Therefore, we concluded that an ICG concentration more than 50 μg/mL (64 μM, 100× dilution of the stock solution) did not improve SLN detection; it was therefore decided to settle for an ICG concentration of 50 μg/mL (64 μM) in the next experiment.

Effect of ICG–Phytate Colloid Mixture on SLN Detection

Figure 3 shows the results of injecting ICG–phytate colloid mixture of different mixing volume ratios. SLN was visible

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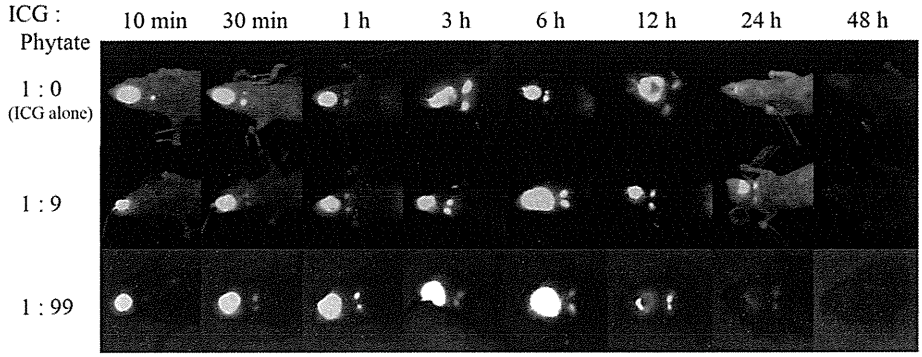

Figure 3. Effect of indocyanine green (ICG)–phytate colloid mixture. Sentinel lymph nodes (SLN) appeared within 30 minutes, peaked at 6 to 12 hours, and remained detectable after 24 hours, with no visualization of second echelon lymph nodes.

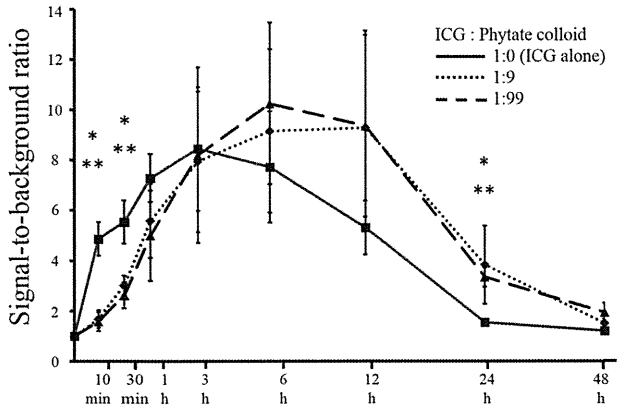

Figure 4. Effect of different ratios of indocyanine green (ICG) and phytate colloid on sentinel lymph nodes (SLN) fluorescence. Fluorescence with ICG–phytate mixtures of both ratios differed significantly from that of ICG at 10 minutes, 30 minutes and 24 hours. *$P < .05$ when compared with ICG alone and ICG:phytate colloid 1:9, **$P < .05$ when compared with ICG alone and ICG:phytate colloid 1:99.

30 minutes after injection in mice injected with ICG–phytate colloid mixture, while SLN was visible right after injection in mice injected with ICG alone. The fluorescent intensity reached its maximum level around 6 to 12 hours after injection. Some SLNs were detectable even after 24 hours, and no second echelon LN was observed in mice injected with ICG–phytate colloid mixture. No obvious toxicity was found in all mice.

Figure 4 shows the change in SBRs measured over time. Signal intensity increased 30 minutes after injection, reached maximum levels around 6 to 12 hours after injection, started decreasing afterwards, but still remained high even after 24 hours in mice injected with ICG–phytate colloid mixture. Significant differences were observed at 10 minutes, 30 minutes, and 24 hours after injection between mice injected with ICG alone and those injected with ICG–phytate colloid mixture in a ratio of 1:9 or 1:99 ($P < .05$). No significant difference was observed at every time point between mice injected with a ICG–phytate colloid mixture with a ratio of 1:9 and 1:99.

The initial detection of SLN was delayed in mice injected with ICG–phytate colloid mixture, but the SLN remained detectable for much longer than in mice injected with plain ICG. These results indicate the advantageous prolongation of the detection time window of SLN with our modified ICG technique.

Detection of Second Echelon Lymph Nodes

Table 1 showed the average number of second echelon LNs detected per mouse. Second echelon LNs were visualized only in mice injected with ICG alone, but not in mice injected with ICG–phytate colloid. These downstream nodes are not visualized with our novel ICG modified technique, which is an advantage because it improves the accuracy of detection of true SLNs.

Discussion

Though SNNS for head and neck region are considered as a technique for mainly melanoma and cutaneous SCC, recent reports for aerodigestive tract HNSCC supports the value of this procedure. Yamauchi et al[25] demonstrated the results of meta-analysis in early HNSCC, in which a total of 16 studies (987 patients) were included. The identification rate, sensitivity, false-negative rate, negative predictive value, and accuracy were 95.2%, 86.3%, 13.7%, 94.2%, and 95.0%, respectively. We have also reported satisfactory results in a retrospective multicenter analysis of 177 patients with oral and laryngopharyngeal SCC in Japan.[26] Fan et al[27] reported the prognosis of cT1-2N0 oral tongue SCC treated with SNNS or elective neck dissection. They concluded that the extent of neck dissection can be reduced for SLN negative patients, owing to the non-inferiority in prognosis associated with SNNS. We have developed trans-oral video-laryngoscopic surgery that enables almost same resection with TORS for oropharynx, hypopharynx, and supraglottis cancer.[11-14] Our experience with 60 patients demonstrated that more than 60% were clinically N0.[14] Therefore SNNS that allows head and neck surgeons to avoid unnecessary neck dissection has great potential

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Table 1. Detection of Second Echelon Lymph Nodes at Various Intervals after Tracer Injection (Number of Second Echelon Lymph Nodes Per Mouse).

	10 min	30 min	1 h	3 h	6 h	12 h	24 h	48 h
Indocyanine green (ICG) alone (5000 μg/mL)	0	0.75	1.25	2	2	1.5	0	0
ICG alone (500 μg/mL)	0	0	0.25	0.75	0.75	0.75	0	0
ICG alone (50 μg/mL)	0	0	0.25	0.5	0.5	0.5	0	0
ICG:phytate 1:9	0	0	0	0	0	0	0	0
ICG:phytate 1:99	0	0	0	0	0	0	0	0

Table 2. Comparison of Plain Indocyanine Green (ICG) with ICG–Phytate Colloid Mixture.

	ICG Alone	ICG–Phytate Colloid
Onset of sentinel lymph nodes (SLN) detection	Right after injection	30–60 min after injection
Time of maximum brightness of SLN fluorescence	3–6 h after injection	6–12 h after injection
SLN detection after 24 h	Difficult	Possible (in many mice)
Visualization of second echelon lymph nodes	Positive	Negative

for establishment of minimally invasive and individualized treatment for HNSCC.

This mouse study showed that our innovative technique of mixing ICG with phytate colloid successfully prolonged the SLN diagnostic time window and prevented downstream nodes from being visualized (**Table 2**). These features of the technique are expected to contribute to true SLN detection, especially in the head and neck region. ICG is injected around the tumor under rigid laryngoscope guidance when SNNS is planned at the same time as trans-oral resection of the primary tumor. Afterwards, the skin is incised over the fluorescent signal, and an attempt is made to detect and biopsy the SLN. These procedures take time, with the consequent risk of losing the true SLN in the midst of many downstream nodes that also fluoresce because of quick migration of ICG. In a preliminary report in patients with cancer of the oral cavity or oropharynx, Van der Vorst et al[28] reported that the SLN could be identified within 5 minutes of injection in 7 out of 10 patients and within 30 minutes in all patients. They also commented that the average number of fluorescent lymph nodes significantly increased over time. In this context, our technique has the potential to prevent the quick migration of ICG and enable true SLN detection with greater assurance.

Phytate colloid is now available as an instant labeling kit in Japan. The simple addition of normal saline containing ^{99m}Tc pertechnetate to this labeling kit yields ^{99m}Tc-phytate colloids with diameters between 100 and 200 nm[29]; the size of colloidal particles grows further upon reaction with ionized calcium in vivo,[30] and these enlarged colloidal particles are preferentially phagocytosed by macrophages. Therefore, ^{99m}Tc-labeled phytate colloids are the tracers of choice for hepatosplenic scintigraphy. It has been reported that the ideal size of tracer particles for SNNS is between 20 and 500 nm[31,32]; ^{99m}Tc-labeled phytate colloids are therefore used for SNNS in Japan. Small particles of ^{99m}Tc-phytate migrate right after injection to SLNs where these combine with calcium ions and increase in size. These larger particles of ^{99m}Tc-phytate with calcium are retained in SLNs for much longer periods without passing through to downstream non-SLN nodes[33]; this property of phytate colloids makes them very suitable for SNNS. A report has demonstrated that colloidal particles tagged to radioactive and non-radioactive labels have a similar distribution in the body.[34] Although we have not confirmed the detailed particle profile of phytate colloid mixed with ICG, our current experimental results suggest that ICG is likely to interact with phytate colloids and that ICG–phytate colloid should be distributed in the similar way as ^{99m}Tc -phytate colloid.

The preparation of ICG–phytate colloid for our technique entails nothing but mixing ICG solution with phytate colloid, a process that is simple and easy. Neither ICG nor phytate colloids are expensive; besides, both have been used with no ethical and safety issues in clinical practice in Japan for a long time. Therefore, ICG–phytate colloid mixture has a high potential for clinical application and it is hoped that attempts will be made to use this technique in a clinical setting in the near future.

It is also planned to demonstrate the use of hybrid ICG-^{99m}Tc-phytate colloid as a fluorescent and radioactive

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tracer at the same time. A similar hybrid tracer technique using nanocolloid for SNNS in the head and neck region has been reported by researchers from the Netherlands who demonstrated that the lymphatic drainage pattern of hybrid ICG-^{99m}Tc nanocolloid was identical to that of ^{99m}Tc-nanocolloid with no adverse reactions.[35,36] These findings warrant further evaluation of hybrid ICG-RN tracer techniques for SNNS to harness the convenience of intraoperative fluorescent guidance after initial RN localization. Our technique using phytate colloid instead of nanocolloid promises to offer the same advantages with the ICG-^{99m}Tc-phytate colloid conjugate migrating slowly through lymphatic channels, resulting in prolonged SLN detection with no visualization of second echelon LNs. Moreover, our technique is cheaper and might possibly be safer because phytate colloids are not proteinaceous in nature, unlike nanocolloids. This novel hybrid technique might therefore have a high potential for clinical application. To the best of our knowledge, there have been no basic studies on hybrid tracers of this kind, and the findings from such research could enhance the role of tracers for SNNS.

Furthermore, we are designing a new treatment strategy to target SLN. ICG is not only a tracer for NIR fluorescence imaging, but also as a sensitizer for photodymanic therapy (PDT) with an absorption maximum of 810 nm.[37] The efficacy of ICG-enhanced PDT has been studied in various fields; however, the strong binding of ICG to plasma proteins leads to rapid clearance in the liver and minimizes selective toxicity for tumors. Side effects related to photosensitivity are also seen after systemic administration.[38] In this context, our technique of conjugating ICG to phytate colloid could enable SLN-specific, targeted PDT by allowing a longer retention time of ICG in the SLN and preventing its migration to lymph nodes further downstream. For example, HNSCC patients without clinically evident lymph node metastasis (N0) undergoing chemotherapy or radiotherapy, can be offered SLN-targeted PDT by injecting ICG–phytate colloid around the tumor and exposing the site of the SLN repeatedly with near-infrared radiation of 810 nm wavelength to take advantage of the prolonged retention of ICG in the SLN. SLN-targeted therapy of this kind should have fewer systemic side effects and is expected to be effective in controlling lymph node metastasis in the head and neck region.

Conclusion

Our modification of the ICG technique for SNNS, consisting of the addition of phytate colloid to ICG, was able to prolong the diagnostic time window of SLNs and prevent the visualization of LNs lying further downstream. This simple and easy technique has the potential to overcome the disadvantage of quick migration of ICG and offer greater assurance in the detection of the true SLN. It also has the advantage of being ready for clinical application without ethical and safety issues. We hope to elucidate and assess the basic mechanism of this technique, apply it in a clinical setting, refine it further as a hybrid fluorescent-RN method, and utilize its potential in SLN-targeted PDT.

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Author Contributions

Koji Araki, designed, collected and analyzed data, wrote and revised article; **Daisuke Mizokami**, collected and analyzed data, revised article; **Masayuki Tomifuji**, designed, revised article; **Taku Yamashita**, designed, revised article; **Kazunobu Ohnuki**, collected and analyzed data, revised article; **Izumi O. Umeda**, designed, collected and analyzed data, revised article; **Hirofumi Fujii**, designed, collected and analyzed data, revised article; **Shigeru Kosuda**, designed, revised article; **Akihiro Shiotani**, designed, revised article.

Disclosures

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ORIGINAL ARTICLE

Impact of positron emission tomography with the use of fluorodeoxyglucose on response to induction chemotherapy in patients with oropharyngeal and hypopharyngeal squamous cell carcinoma

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Abstract

Conclusion: Maximum standardized uptake values (SUVmax) have prognostic value for induction chemotherapy (ICT) response and survival in oropharyngeal and hypopharyngeal squamous cell carcinoma (OHSCC) patients. Pretreatment positron emission tomography with the use of fluorodeoxyglucose (^{18}F-FDG PET) may be an aid in deciding the treatment strategy in OHSCC patients. *Objectives:* We investigated the association between pretreatment ^{18}F-FDG PET and response to ICT and survival in OHSCC patients. *Methods:* We conducted a retrospective cohort study of 58 OHSCC patients treated at Aichi Cancer Center Hospital. The predictive impact of SUVmax of the primary tumor site was evaluated using statistical multivariate proportional hazard models. *Results:* Thirty-one cases (53%) were located in the oropharynx and 27 (47%) in the hypopharynx. Median SUVmax was 11.6 (range 3.2–23.5), and was significantly higher in the 8 patients with less than stable disease than in the 50 with more than partial response (median SUVmax, 17.3 vs 11.1; $p = 0.002$). In multivariate analysis, hazard ratios for the medium and high SUVmax groups relative to the low group were 3.07 (95% confidence interval, 0.62–15.29; $p = 0.170$) and 4.71 (0.97–22.89; $p = 0.055$), respectively, and the dose-response relationship was statistically significant ($p_{trend} = 0.047$). A similar tendency was observed on subclassification by oropharynx and hypopharynx.

Keywords: *Oropharyngeal cancer, hypopharyngeal cancer, survival, PET*

Introduction

Cancers of the head and neck, which typically arise from epithelial squamous cells, are the sixth most common malignancies worldwide, and over 398 000 new oral and pharyngeal cancers and 151 000 new laryngeal cancers were estimated to have arisen globally in 2008 [1]. In Japan, the incidence of oral and pharyngeal cancers has increased steadily from 1960 to 2000 [2]. Approximately 60% of head and neck squamous cell carcinomas (HNSCCs) are detected at an advanced stage (stage III–IV), and prognosis of these cases has remained markedly poor over the last two decades despite the advent of multimodal treatment [3,4]. Although prognostic factors have been established, namely primary tumor site, TNM classification, and comorbidity [5], the identification of additional prognostic factors might lead to more effective personalized treatment in the management of HNSCC.

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Positron emission tomography with the use of the radiolabeled glucose analog 18F-fluorodeoxyglucose (^{18}F-FDG PET) has recently been included as a routine imaging modality to conventional imaging examinations before treatment, such as computed tomography (CT) and magnetic resonance imaging (MRI), in the management of HNSCC. A semiquantitative measure of the FDG uptake of cancers is usually obtained by the maximum standardized uptake values (SUVmax). Several reports have evaluated the association between SUVmax and clinical outcome in several cancers, including HNSCC [6–13]. We also previously reported the prognostic value of PET in patients with oral cavity cancer [11]. However, the association between clinical outcome and SUVmax in HNSCC has remained controversial.

Additionally, although many institutions, including ours, have used induction chemotherapy (ICT) for organ preservation treatment in oro- and hypopharyngeal squamous cell carcinoma (OHSCC) patients, the association between FDG-PET and response to ICT in OHSCC has been scarcely reported.

Here, we conducted a retrospective cohort study to evaluate the association between SUVmax and response to ICT or clinical outcome in OHSCC patients.

Material and methods

Patients

The study was conducted under a retrospective cohort design in patients with OHSCC treated at Aichi Cancer Center Hospital between January 2004 and February 2007. Principal inclusion criteria were a histological diagnosis of squamous cell carcinoma, no distant metastasis, and provision of written informed consent. Fifty-eight OHSCC patients were enrolled and their medical records were reviewed.

Treatment and follow-up

All tumors were staged according to the 2002 Union for International Cancer Control (UICC) tumor-node-metastasis staging system. In OHSCC patients, surgery with wide excision results in problems in swallowing and speech. We therefore performed induction chemotherapy (ICT) for organ preservation. After ICT, we diagnosed the response to ICT by the RECIST criteria using physical and conventional imaging examination, which included CT or MRI. Responders who were evaluated as having partial response (PR) or complete response (CR) were recommended for concurrent chemoradiotherapy (CCRT), whereas non-responders who were diagnosed with stable disease (SD) or progressive disease (PD) were recommended for definitive surgery. Following the end of treatment, patients underwent a history and physical examination, complete blood cell count, and imaging examination every 3–6 months for 5 years. Vital and disease status were confirmed by checking medical records at the date of the last follow-up visit.

FDG-PET scan

All patients were scanned using an FDG-PET scan (Advance NXi; GE, Fairfield, USA) at the Nagoya PET Imaging Center within 4 weeks before treatment. After fasting for 6 h, patients were given an intravenous injection of 5–10 mCi of FDG, sat quietly for 60 min, and were then scanned from the mid-thigh to the skull. Transmission data for attenuation correction were acquired after the emission scan. Images were reconstructed using a 2-dimensional ordered subset expectation maximization algorithm with 28 subsets and 4 iterations. The Gaussian-based 6 mm full-width at half-maximum (FWHM) post-filter and 4.3 mm FWHM loop filter were used during reconstruction. All images were viewed on a Xeleris (GE, USA) workstation by two experienced radiologists, who compiled all the reports. A focus was considered positive if activity was significantly above the expected background and could not be explained by a normal structure.

A whole-body coronal image was made in SUV mode for semiquantitative evaluation. FDG uptake was calculated as SUV by the following formula: SUV = tissue concentration (Bq/g)/injection dose (Bq)/body weight (g). The maximum SUV (SUV-max) of a primary tumor site was obtained from a region of interest, which was defined as a site of abnormal accumulation on the coronal image.

Statistical analysis

The primary end point was the response rate (RR) to ICT (PR + CR vs SD + PD). The association between RR and SUVmax was evaluated by the Mann–Whitney test. The second end point was overall survival (OS), defined as the interval between the beginning of treatment and the date of death or last follow-up. The association between SUVmax and OS was evaluated by the Kaplan–Meier product-limit method and univariate and multivariate Cox proportional hazards models. The measure of association in this study was hazard ratio (HR) with a 95% confidence interval (CI). Confounders considered in the univariate and multivariate analyses were age (continuous variable), sex (male vs female),

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T classification (1/2/3/4), N classification (0/1/2/3), and primary tumor site (oropharynx vs hypopharynx).

All statistical analyses were performed using STATA version 10 (Stata Corp., College Station, TX, USA). All tests were two-sided, and p values of < 0.05 were considered statistically significant.

Results

Patients' characteristics and survival

Table I summarizes the characteristics of the 58 OHSCC patients evaluated in this study. Median age was 59 years (range 36–76 years) and median follow-up time was 4.6 years (range 0.4–7.0 years).

Table I. Patients' characteristics.

Characteristic	*n*	%
Age (years), median (range)	59 (36–76)	
Sex		
Male	51	88
Female	7	12
Primary tumor site		
Oropharynx	31	53
Hypopharynx	27	47
T classification		
1	3	5
2	30	52
3	19	33
4	6	10
N classification		
0	16	28
1	4	7
2	35	60
3	3	5
UICC stage		
1	1	2
2	9	15
3	10	17
4	38	66
Response to induction chemotherapy		
SD/PD	8	14
PR/CR	50	86
SUVmax		
Low (< 10)	19	33
Medium (≥ 10 and < 14.2)	20	34
High (≥ 14.2)	19	33

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; SUVmax, maximum standardized uptake values; UICC, Union for International Cancer Control.

Males were predominant (88%). Primary tumor site was the oropharynx in 31 cases (53%) and hypopharynx in 27 (47%). Regarding clinical disease stage, T2, N2 (2a/2b/2c), and UICC stage 4 (4a/4b) were most common. A response to ICT was seen in 8 non-responders (14%) and in 50 responders (86%). Stratified by response to ICT, the distribution of T classification was significantly different between responder and non-responders ($p = 0.003$, Supplementary Table I). ICT regimens were as follows: cisplatin 80 mg/m^2 on day 5, 5-fluorouracil (5-FU) 800 mg/m^2 on days 1–5, every 3 weeks ($n = 45$); and cisplatin 25 mg/m^2 on day 1 and 5-FU 1000 mg/m^2 on days 1–2, weekly ($n = 13$). ICT and CCRT schedules are detailed in Supplementary Table II. The 5-year OS among all patients was 73.2% (95% CI, 59.5–82.9).

Association between SUVmax and response to ICT

The median SUVmax was 11.6 (range 3.2–23.5). Figure 1 shows the association between SUVmax and response to ICT using a box plot. Response to ICT was significantly associated with pretreatment SUVmax (median SUVmax: 17.3 in non-responder vs 11.1 in responder; $p = 0.002$).

The cut-off value for SUVmax between responder and non-responder in OHSCC patients was evaluated from the sensitivity and specificity of the serial cut-off values. In this study, detection was optimum at a cut-off of 12.21 (sensitivity 100.0%, specificity 62.0%).

Figure 2 shows these associations by primary tumor site. A similar tendency was observed with statistical significance among oropharyngeal and hypopharyngeal disease (median SUVmax: oropharynx, 17.5 in non-responders vs 11.9 in responders, $p = 0.034$; hypopharynx, 16.0 in non-responders vs 10.6 in responders, $p = 0.017$).

Impact of SUVmax on survival

In the absence of an established cut-off for SUVmax value, estimated SUVmax was divided into three groups, i.e. low (<10, lowest tertile), medium (≥ 10 and <14.2, middle tertile), and high (≥ 14.2, highest tertile). The number of patients in these groups was 19, 20, 19, respectively.

Figure 3 shows 5-year OS according to SUVmax. Five-year OS was 88.8% (95% CI, 62.1–97.1) for low, 68.9% (43.3–84.7) for medium, and 62.4% (36.7–80.0) for high SUVmax (log rank test, $p = 0.148$), showing that a high SUVmax was associated with a lower OS than a low and medium SUVmax. Additionally, these associations were

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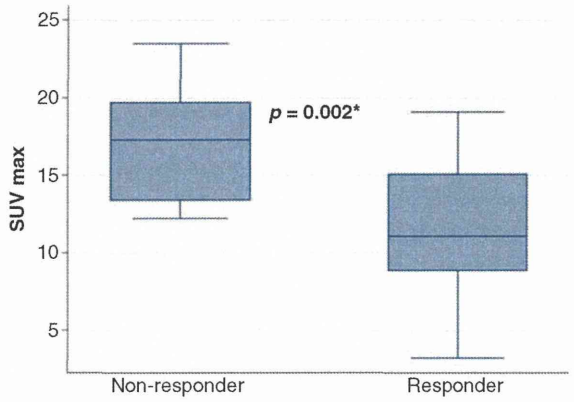

Figure 1. Association between response to induction chemotherapy and pretreatment maximum standardized uptake values (SUVmax). Median SUVmax, 17.3 in non-responder vs 11.1 in responder, $p = 0.002$. *Mann–Whitney test.

maintained when oropharyngeal and hypopharyngeal cases were evaluated separately (Figure 4). Among oropharyngeal squamous cell carcinoma patients, 5-year OS was 87.5% (95% CI, 38.7–98.1) for low, 88.9% (95% CI, 43.3–98.4) for medium, and 75.0% (95% CI, 40.8–91.2) for high SUVmax ($p = 0.527$). Among hypopharyngeal squamous cell carcinoma patients, the 5-year OS was 88.9% (95% CI, 43.3–98.4) for low, 49.9% (95% CI, 17.3–75.9) for medium, and 42.9% (95% CI, 9.8–73.4) for high SUVmax ($p = 0.157$).

Table II shows the results of univariate analysis of SUVmax for OS. HRs for the medium and high SUVmax groups relative to the low group were 3.10 (95% CI, 0.63–15.37; $p = 0.166$) and 4.31 (95% CI, 0.89–20.77; $p = 0.069$), respectively, and the dose-response relationship was marginally statistically significant ($p_{trend} = 0.062$). Additionally, T classification and primary tumor site remained statistically significant.

Table III shows the results of the multivariate analysis of SUVmax for OS. We evaluated the correlation between SUVmax and T classification using Spearman correlation models and found a moderate correlation (Spearman's coefficient = 0.510). SUVmax, T classification (T1–3 vs T4), N classification (N0 vs N1–3), and primary tumor site were selected for multivariate analysis. HRs for the medium and high SUVmax groups relative to the low group were 3.07 (95% CI, 0.62–15.29; $p = 0.170$) and 4.71 (95% CI, 0.97–22.89; $p = 0.055$), respectively, and the dose-response relationship was statistically significant ($p_{trend} = 0.047$). Additionally, T4 was significantly associated with poor survival.

Finally, we also performed stratified analyses by T classification and primary tumor site (Table IV). The effect of SUVmax on survival was not affected by T classification or primary tumor site.

Discussion

In this study, we demonstrated that pretreatment SUVmax of the primary tumor site might predict response to ICT and survival in patients with OHSCC. Additionally, this study validated our previous finding in patients with oral cavity cancer that

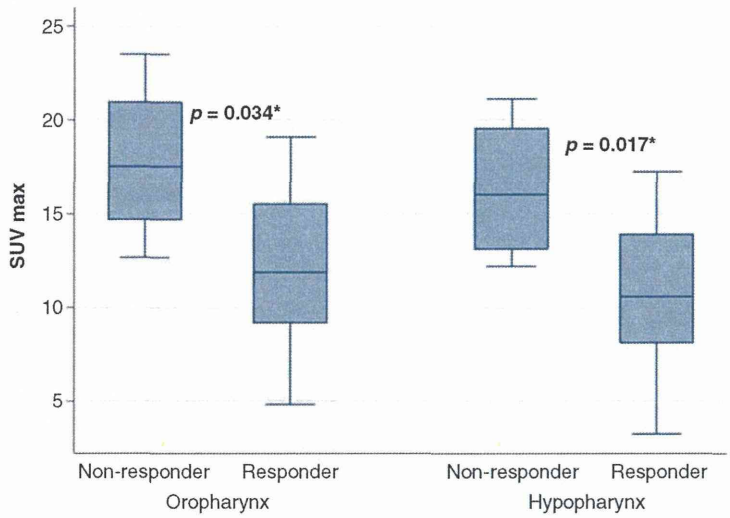

Figure 2. Association between response to induction chemotherapy and pretreatment maximum standardized uptake values (SUVmax) according to primary tumor site. Median SUVmax: oropharynx, 17.5 in non-responder vs 11.9 in responder, $p = 0.034$; hypopharynx, 16.0 in non-responder vs 10.6 in responder, $p = 0.017$). *Mann–Whitney test.

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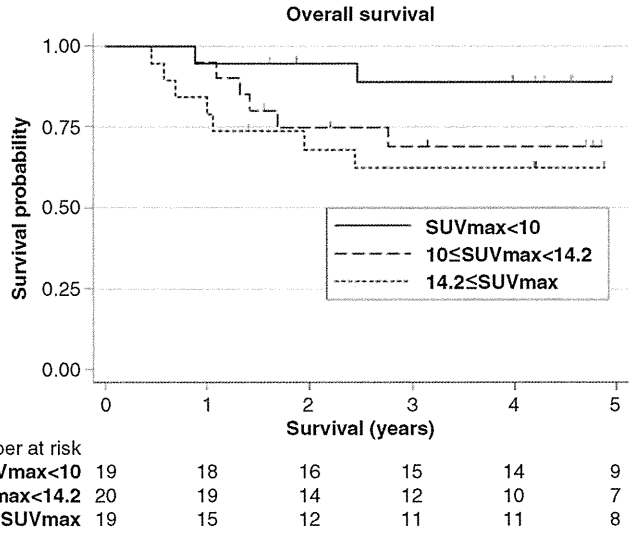

Figure 3. Kaplan–Meier survival curves for maximum standardized uptake values (SUVmax). Five-year overall survival (OS) was 88.8% (95% confidence interval, 62.1–97.1) for low, 68.9% (43.3–84.7) for medium, and 62.4% (36.7–80.0) for high SUVmax (log rank test, $p = 0.148$).

indicated a prognostic value of SUVmax. To our knowledge, this study represents the first evaluation of the association between SUVmax and ICT response in HNSCC patients.

Although the mechanisms behind this association between pretreatment FDG-PET and ICT response and survival in OHSCC patients is unclear, previous evidence indicates several possibilities. First, the

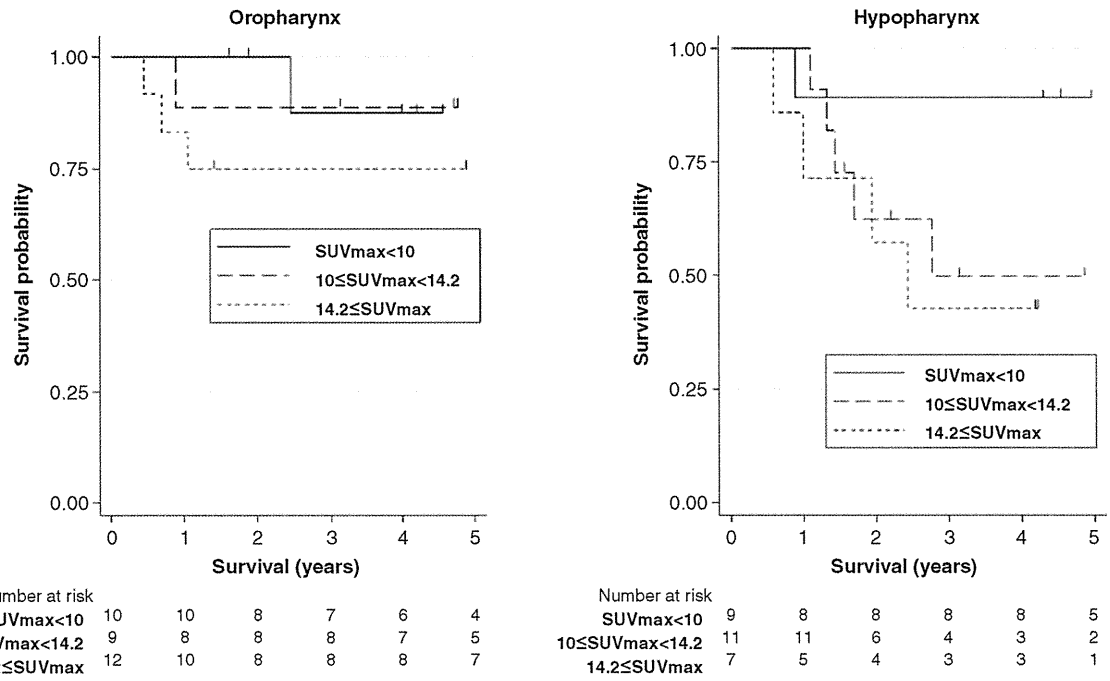

Figure 4. Kaplan–Meier survival curves for maximum standardized uptake values (SUVmax) according to primary tumor site. Among oropharyngeal squamous cell carcinoma patients, 5-year overall survival (OS) was 87.5% (95% confidence interval (CI), 38.7–98.1) for low, 88.9% (43.3–98.4) for medium, and 75.0% (40.8–91.2) for high SUVmax (log rank test, $p = 0.527$). Among hypopharyngeal squamous cell carcinoma patients, 5-year OS was 88.9% (95% CI, 43.3–98.4) for low, 49.9% (17.3–75.9) for medium, and 42.9% (9.8–73.4) for high SUVmax (log rank test, $p = 0.157$).

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Table II. Univariate analysis of survival in oropharyngeal and hypopharyngeal cancer patients.

	Overall survival		
Variable	HR	95% CI	*p* value
SUVmax			
Low (< 10)	1.00	Reference	–
Medium (≥ 10 and < 14.2)	3.10	0.63–15.37	0.166
High (≥ 14.2)	4.31	0.89–20.77	0.069
p_{trend}			0.062
T classification			
1	1.00	Reference	–
2	0.28	0.03–2.71	0.272
3	1.17	0.14–9.50	0.885
4	3.73	0.41–33.50	0.240
p_{trend}			0.002
N classification			
0	1.00	Reference	–
1	2.19	0.20–24.23	0.522
2	2.88	0.65–12.89	0.165
3	Not estimated	–	–
p_{trend}			0.346
Primary tumor site			
Oropharynx	1.00	Reference	–
Hypopharynx	2.43	0.83–7.11	0.106
Response to induction chemotherapy			
SD/PD	1.00	Reference	–
PR/CR	0.23	0.08–0.68	0.008

CI, confidence interval; CR, complete response; HR, hazard ratio; PD, progressive disease; PR, partial response; SD, stable disease; SUVmax, maximum standardized uptake values.

Table III. Multivariate analysis of survival in oropharygeal and hypopharyngeal cancer patients.

	Overall survival		
Variable	HR	95% CI	*p* value
SUVmax			
Low (< 10)	1.00	–	–
Medium (≥ 10 and < 14.2)	3.34	0.67–16.76	0.143
High (≥ 14.2)	6.62	1.24–35.41	0.027
p_{trend}			0.022
T classification			
1–3	1.00	Reference	–
4	23.67	4.56–122.76	< 0.001
N classification			
0	1.00	Reference	–
1–3	1.15	0.23–5.63	0.863
Primary tumor site			
Oropharynx	1.00	–	–
Hypopharynx	2.52	0.83–7.62	0.102

CI, confidence interval; HR, hazard ratio; SUVmax, maximum standardized uptake values.

Warburg effect is a characteristic of cancer in which malignant tumors are characterized by higher glucose metabolism than healthy cells [14,15]. FDG is transported into the cytoplasm and phosphorylated to become FDG-6-phosphatate. It is not metabolized further, and remains inside the cells [16]. FDG uptake is therefore used as an indicator of glucose metabolism. Because more aggressive tumors need more energy, including glucose, these malignancies might show a higher SUVmax. Second, it has been reported that the Glut family of glucose transporter genes is overexpressed in carcinogenesis [17]. It is therefore plausible that the measurement of parameters relating to glucose transport within tumor cells may be of value in predicting response to cancer treatment. Previously, however, we were unable to observe a significant association between Glut-1 and prognosis in patients with oral cavity cancer [11]. Third, we previously reported that SUVmax might correlate with the expression of Bcl-2, an anti-apoptic protein, in patients with oral cavity cancer. Friedman et al. suggested that high expression of Bcl-2 is associated with poor prognosis in head and neck cancer [18].

Although it has been known that the management of neck was an important predictor in HNSCC patients, N classification was not significantly associated with survival in OHSCC patients in this study. Actually, N classification has a decreased trend for survival in univariate analysis, although not in multivariate analysis. These results might be derived from the interaction between N classification and SUVmax, which indicate tumor progression and invasion [19].

In addition, the association between pretreatment FDG-PET and prognosis remains controversial in HNSCC patients. Recently, Omloo et al. reviewed the association between FDG-PET and survival in esophageal cancer patients. They mentioned that several different normalization factors for FDG uptake are in use and more research focusing on standardization of protocols and inter-institutional differences should be performed [20]. Their opinions could be applied in HNSCC patients.

The present study has one methodological strength: clinicians involved in the care of study patients had no information on the association between FDG-PET and HNSCC survival, which likely precluded the introduction of information bias.

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Table IV. Impact of SUVmax on survival stratified by T classification and primary tumor site in oropharyngeal and hypopharyngeal cancer patients.

		Overall survival		
Factor	Variable	HR	95% CI	*p* value
T classification				
T1–3	SUVmax	1.91	0.85–4.32	0.119
T4	SUVmax	2.78	0.38–20.59	0.316
Primary tumor site				
Oropharynx	SUVmax	3.47	0.75–15.99	0.111
Hypopharynx	SUVmax	1.82	0.75–4.43	0.186

Adjusted by N classification. CI, confidence interval; HR, hazard ratio; SUVmax, maximum standardized uptake values.

Several limitations of this study warrant mention. First, our information about PET reflected pretreatment status only. Several studies have reported that PET after definitive treatment might have predictive value. Second, the moderate sample size may have limited the study, necessitating the duplication of this work in another cohort. Finally, we could not adjust for the effect of HPV infection in oropharyngeal cancer patients. In this regard, however, results for oropharyngeal and hypopharyngeal cancer by stratified analysis were similar.

In conclusion, we found that pretreatment FDG-PET has predictive value for response to ICT and survival in patients with OHSCC. These findings may be useful in the establishment of personalized treatment for organ preservation in OHSCC patients. Further study to validate our results is warranted.

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Supplementary material available online

Supplementary Table I ,Table II

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ORIGINAL ARTICLE

The contribution of neck dissection for residual neck disease after chemoradiotherapy in advanced oropharyngeal and hypopharyngeal squamous cell carcinoma patients

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Abstract
Background Planned neck dissection after chemoradiotherapy (CRT) has remained controversial in advanced oro- and hypopharyngeal squamous cell carcinoma (OHSCC) patients. We evaluated the survival contribution of neck dissection (ND) in OHSCC patients with residual nodal disease following CRT.
Methods We retrospectively evaluated 84 OHSCC patients with N2–3 disease treated at Aichi Cancer Center Hospital between 1995 and 2006. ND after CRT was performed for residual neck disease in 36 patients, but not in 48 patients to achieve a complete response. These two groups were analyzed in terms of both overall survival (OS) and regional control (RC), and surgical complications were evaluated.
Results The 5-year OS was 76.7 % [95 % confidence interval (CI) 58.8–87.6] for the ND group and 73.9 % (58.6–84.3) for the non-ND group ($P = 0.883$). The 5-year RC was 91.6 % (76.1–97.2) for the ND group and 81.1 % (65.4–90.2) for the non-ND group ($P = 0.252$). Stratified by primary tumor site, the 5-year RC was 96.3 % (76.5–99.5) for the ND group, and 78.6 % (58.0–89.9) for the non-ND group ($P = 0.072$) in oropharyngeal squamous cell carcinoma patients, and 77.8 % (36.5–93.9) for the ND group and 85.9 % (54.0–96.3) for the non-ND group ($P = 0.541$) in hypopharyngeal squamous cell carcinoma patients. In addition, the complications after ND were tolerable.
Conclusions We demonstrated that ND was feasible, safe, and correlated with clinical outcomes in OHSCC patients with residual nodal disease after CRT.

Keywords Head and neck cancer · Chemoradiotherapy · Neck dissection · Survival

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Introduction

Head and neck cancers typically arise from epithelial squamous cells, with more than 500,000 new cases diagnosed worldwide each year [1]. In Japan, mortality due to pharyngeal cancers increased steadily from 1960 to 2000, reaching levels observed in Western countries by the 1990s [2]. Approximately 60 % of patients present with advanced disease (stages III and IV), for which the prognosis is poor even with multimodal treatment approaches [3]. The established prognostic factors are primary tumor site, TNM stage, and comorbidity, including performance status (PS) [4], and it is well known that neck-disease control is more important for the effective management of head and neck cancer.

Planned neck dissection (PND) was originally performed regardless of the nodal response after definitive radiotherapy (RT) in advanced head and neck squamous

cell carcinoma (HNSCC) patients. PND was recommended on the basis of a report that the regional control rates of RT with neck dissection (ND) were higher than those of RT alone in patients with N2–3 neck disease [5]. However, other investigators have reported that ND should be performed only if clinical or radiographic evidence of residual neck disease is present [6–8], and the effects of PND have remained controversial. In addition, concurrent chemoradiotherapy (CRT) has been a first-line treatment among the therapeutic methods for HNSCC [9]. Since patients with N1 disease have high regional control rates, they do not require ND unless there is evidence of persistent disease after definitive CRT [10]. The role of PND after definitive CRT has become more controversial in HNSCC patients who have N2–3 disease.

In our institute, CRT has been performed as the first-line treatment for organ preservation in the management of advanced oro- and hypopharyngeal squamous cell carcinoma (OHSCC) patients. Following CRT, we diagnosed treatment responses using physical and imaging examinations after 2–4 weeks. If residual neck disease was suspected, we performed ND after 4–8 weeks.

In this study, we aimed to evaluate the feasibility and survival contribution of ND in advanced OHSCC patients with residual nodal disease following definitive CRT. To address this issue, we conducted a retrospective cohort study examining this association among 84 OHSCC patients treated at the Aichi Cancer Center Hospital in Nagoya, Japan.

Patients and methods

We retrospectively evaluated 84 OHSCC patients with N2–3 disease treated between 1995 and 2006 at the Aichi Cancer Center Hospital (ACCH). This study was approved by the Institutional Ethics Review Board of ACCH. Staging was performed according to the American Joint Committee on Cancer staging guidelines [11]. All patients were treated with platinum-based concomitant CRT. The majority received a 24-h continuous infusion of 5-fluorouracil (5-FU) (600 mg/m^2 per day) on days 1–6, and cisplatin (80 mg/m^2) on day 7. Four patients received a 24-h continuous infusion of 5-FU (800 mg/m^2 per day) on days 1–5, and nedaplatin (130 mg/m^2) on day 6. These were repeated every 3–4 weeks, and a total of 2 or 3 cycles were performed. Four patients received an infusion of cisplatin (25 mg/m^2) on day 1, followed by a 24-h continuous infusion of 5-FU (1,000 mg/m^2 per day) on days 1–2. This was repeated every week until a total of 6 cycles were performed. Definitive radiotherapy was performed with a conventional fraction (2 Gy/fraction, once a day and five times a week: median 66.6 Gy, range 50.4–72.2 Gy).

For the assessment of treatment response, imaging examinations (computed tomography or magnetic resonance imaging) were performed within 2–4 weeks after CRT. Using both physical and imaging examinations, we decided whether residual diseases were or were not present using Response Evaluation Criteria In Solid Tumors (RECIST). When residual neck diseases were suspected, we performed ND within 4–8 weeks after CRT.

After the end of each treatment, a history and physical examination, complete blood cell count, and imaging examination were performed approximately every 3–6 months for 5 years or more. Vital and disease status were confirmed by checking medical records on the date of the last follow-up visit.

The primary endpoint of this study was overall survival (OS), which was defined as the interval between the beginning of treatment and the date of death or last follow-up. The second endpoint of this study was regional control (RC), which was defined as the interval between the beginning of treatment and the date of neck relapse or last follow-up and the evaluation of surgical complications. The associations between ND and OS or RC were evaluated using the Kaplan–Meier product-limit method and uni- and multivariate Cox proportional hazard models. A measure of such associations in this study was the hazard ratio (HR) along with the 95 % confidence interval (CI). The distribution of patients' characteristics was assessed by the χ^2 test or Fisher's exact test, whichever was more appropriate. All statistical analyses were performed using the software STATA ver. 10 (StataCorp, College Station, TX, USA). All tests were two-sided, and P values <0.05 were considered statistically significant.

Results

Patient characteristics and clinical outcomes

Table 1 summarizes the detailed characteristics of the 84 OHSCC patients evaluated in this study. The primary tumor site involved 59 patients (70 %) for oropharynx, and 25 patients (30 %) for hypopharynx. Median follow-up time was 5.8 years (range 0.6–16.7 years). All patients achieved a clinical CR at the primary tumor site. ND was performed for 36 OHSCC patients (42.9 %), whereas ND was not performed for 48 patients (57.1 %) who were not clinically judged to have residual nodal disease. Regarding the extent of ND, although three hypopharyngeal cancer patients underwent jugular and posterior ND, almost all cases underwent total ND. Of 36 patients with ND, 31 (86.1 %) were found to have pathologically residual malignant cells within their neck nodes. However, we did not observe any pathologically positive lymph nodes