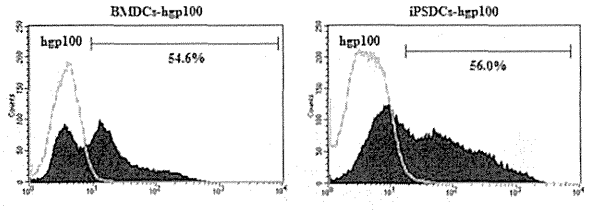

Figure 3. Expression of hgp100 in the genetically modified DCs. The intracellular expression of hgp100 in the genetically modified BMDCs and iPSDCs. DCs were analyzed using intracellular staining flow cytometry. The staining patterns of hgp100 (black) and FITC-matched controls (thin lines) are shown in histograms.

Briefly, B16, MC38 and YAC-1 were used as target cells. The target cells, labeled with $Na_2{}^{51}CrO_4$, were incubated in triplicate with effector cells at various E/T ratios at 37°C in a 5% CO_2 atmosphere for 4 hr. The supernatant was harvested, and the level of radioactivity was counted using a gamma counter. The maximum amount of ^{51}Cr incorporated in the supernatant was determined by adding 1 N HCl to the target cells. The percentage of cytotoxicity was calculated as follows: percentage of lysis = [(sample cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm)] × 100. The lymph node cells were removed and the *in vivo*-primed lymph node cells were restimulated with mitomycin C-treated B16 cells in the same way as described above. After 5 days of coculture, the *in vivo*-restimulated lymph node cells were assayed in a 4-hr ^{51}Cr-release assay with B16 and MC38 as target cells, as described above.

Experimental design of in vivo tumor therapy for the subcutaneous tumor model

To assess whether pre-existing subcutaneous tumors could be suppressed following immunization with DCs (BMDCs or iPSDCs) genetically modified to express hgp100, C57BL/6 mice were immunized *via* subcutaneous injection in the right flank with B16 cells (1.0×10^6 cells), as previously described.[5,6] On Day 5, the tumor-bearing mice ($n = 5$ mice/group) were treated with subcutaneous injections in the opposite flank with genetically modified DCs (1.0×10^6 cells). The volume of the subcutaneous tumors was estimated every 2 or 3 days using the following formula: (short diameter)^2 × long diameter × 0.52.

Statistical analysis

The SPSS software program ver. 18.0 (SPSS, Chicago, IL) was used for all statistical analyses. Quantitative results were expressed as the mean ± SD. Two-tailed Student's *t*-test was used to determine the statistical significance of differences, and a *p*-value of < 0.05 was considered to be significant.

Results

Generation of DCs from iPS cells

Undifferentiated iPS cells were maintained on the feeder layers of SNL cells. To initiate differentiation, the iPS cells were transferred onto OP9 feeder layers. On Day 3, mesodermally differentiated flat colonies appeared. On Day 7, most of the colonies exhibited a differentiated morphology, and the cells were harvested. Subsequently, the cells were transferred onto new OP9 feeder layers and cultured with rmGM-CSF to start Step 2. In Step 2, homogenous small cells resembling primitive hematopoietic stem cells appeared. The iPS cell-derived round cells gradually increased and became morphologically heterologous. Because the addition of rmGM-CSF was essential for the proliferation of the cells, we changed the medium every 2 or 3 days in Step 2. On Day 14, the floating or loosely adherent cells were recovered *via* pipetting. At the beginning of Step 3, we transferred the cells into bacteriological Petri dishes without feeder cells. On Day 26, most of the floating cells, that is, the immature iPSDCs, exhibited an irregular shape with areas of protrusion. The cells were then cultured with 1,000 units/ml of rmGM-CSF and 5,000 units/ml of rmTNF-α for 2 days in Step 4. Finally, on Day 28, the mature iPSDCs were collected and found to be morphologically similar to mature BMDCs (Fig. 1*a*). The yield of differentiation cells was 440 times the cell number in Step 1, 1.35 times the cell number in Step 2 and 1.05 times the cell number in Step 3. Subsequently, the total yield was more than 600 times from the iPS cells to the iPSDCs.

Expressions of DC surface markers

A flow cytometric analysis demonstrated that the immature iPSDCs exhibited high levels of the cell surface expression of CD11c only. In the mature iPSDCs, however, high levels of the cell surface expression of CD11c, CD80, CD86 and MHC Class II were noted. All cell surface expression ratios in the mature iPSDCs were higher than those observed in the immature iPSDCs, as was also the case in the BMDCs (Fig. 1*b*). All experiments were performed five times to confirm the reproducibility of the results, and similar results were obtained each time.

Secretion of IL-12 and IFN-γ from DCs

Neither immature BMDCs nor iPSDCs secreted IL-12 or IFN-γ. The levels of secretion of IL-12 and IFN-γ by mature BMDCs and iPSDCs were significantly higher than those of the immature DCs (both $p < 0.0001$, Fig. 1*c*). Furthermore, there were no significant differences in IL-12 or IFN-γ secretion between the mature BMDCs and iPSDCs ($n = 5$, $p > 0.05$).

Migratory capacity of DCs in vivo

To evaluate the migratory capacity of the BMDCs and iPSDCs *in vivo*, we compared the *in vivo* function. First, the expression of cell surface CCR7 was analyzed using a flow cytometric analysis. The percentage of positively stained cells was less than 10% in the immature BMDCs and iPSDCs, whereas the percentage in the mature BMDCs and iPSDCs rose to 30% and 41%, respectively (Fig. 2*a*). Next, BMDCs-hgp100 and iPSDCs-hgp100 cells were stably labeled with a

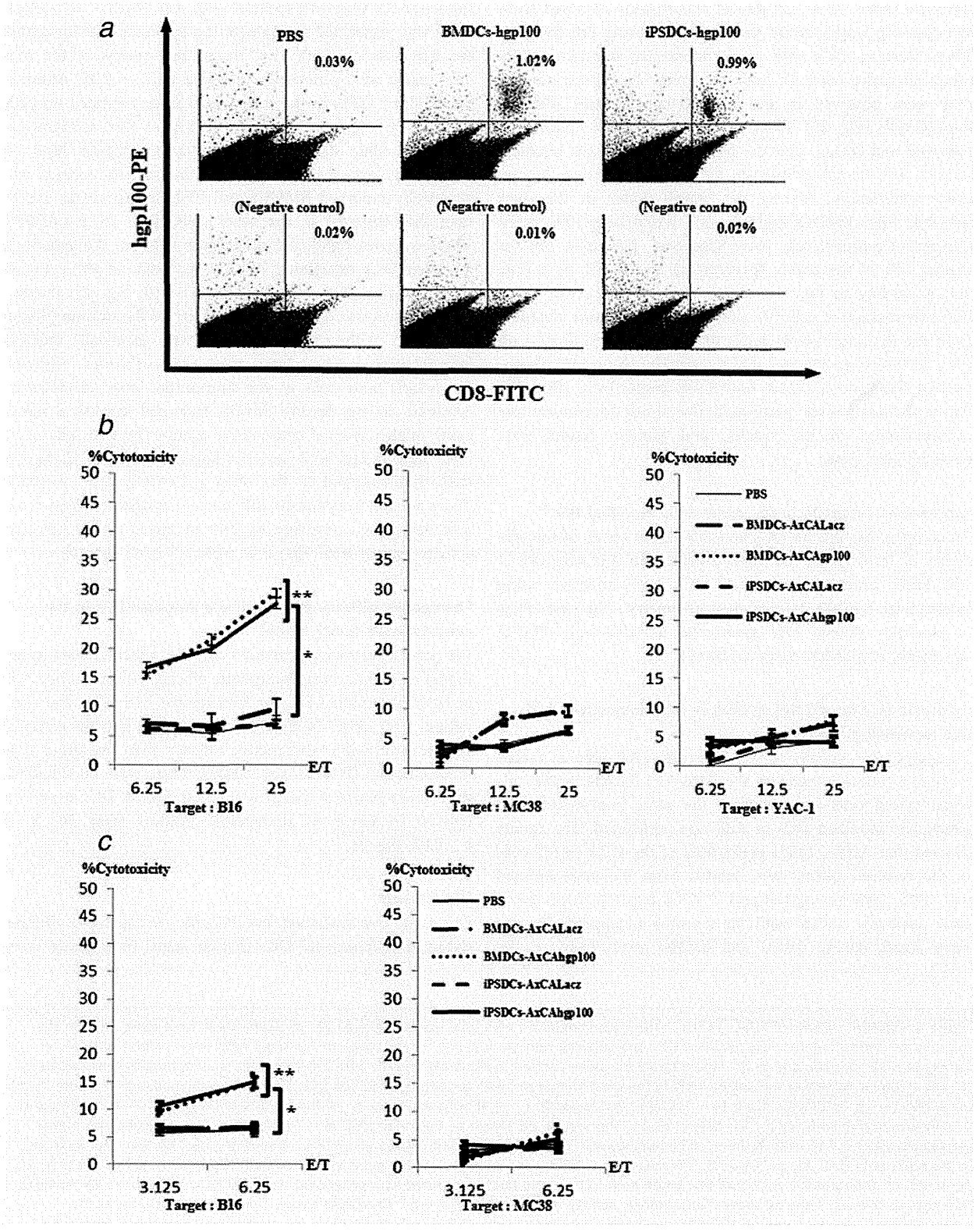
a
PBS
0.03%
BMDCs-hgp100
1.02%
iPSDCs-hgp100
0.99%
(Negative control)
0.02%
(Negative control)
0.01%
(Negative control)
0.02%
hgp100-PE
CD8-FITC
b
%Cytotoxicity
E/T
6.25
12.5
25
Target : B16
Target : MC38
Target : YAC-1
PBS
BMDCs-AxCALacz
BMDCs-AxCAgp100
iPSDCs-AxCALacz
iPSDCs-AxCAhgp100
c
3.125
6.25
Target : B16
Target : MC38

Figure 4.

fluorescent dye (PKH67) and subcutaneously injected into the mice. After 72 hr, single-cell suspensions obtained from the draining lymph nodes were analyzed using cell imaging. There were no DCs with green fluorescent dye in the PBS group (negative control); however, green fluorescent-positive cells were observed in the BMDCs-hgp100 and iPSDCs-hgp100 cells (Fig. 2*b*). Then, the draining lymph nodes were removed and frozen, and the frozen tissue sections counterstained with hematoxylin/eosin were observed using fluorescence microscopy. Among the lymph nodes in the mice injected with BMDCs-hgp100 and iPSDCs-hgp100, green fluorescent-positive cells were observed primarily on the medial side of the cortex (paracortex) and T-cell zone (Fig. 2*c*). According to flow cytometry, the populations of green fluorescent-positive cells in single-cell suspensions obtained from the draining lymph nodes of the mice immunized with PBS, BMDCs-hgp100 and iPSDCs-hgp100 were 3.0% (background fluorescence), 11% and 9.9%, respectively (Fig. 2*d*). All experiments were performed five times to confirm the reproducibility of the results, and similar results were obtained each time.

Expression of hgp100 in the genetically modified DCs

To compare mature BMDCs and iPSDCs in terms of the efficiency of transfecting the hgp100 gene with AxCAhgp100 at 100 MOI, genetically modified DCs were analyzed using intracellular hgp100-staining flow cytometry. The percentage of positively stained cells among the BMDCs and iPSDCs was 54.6% and 56.0%, respectively (Fig. 3).

Cytotoxic activity of CD8(+) CTLs in mice immunized with DCs expressing hgp100

To analyze the capacity of DCs to prime TAA-specific T-cells *in vivo*, a tetramer assay of the CD8 (+) T-cells in the cultured spleen cells isolated from the mice immunized with genetically modified DCs or PBS was performed. The results showed that 0.03%, 1.02% and 0.99% of the CD8 (+) T-cells in the cultured spleen cells isolated from the mice injected with PBS, BMDCs-hgp100 and iPSDCs-hgp100, respectively, were positively stained with the tetramer of hgp100. On the other hand, 0.02%, 0.01% and 0.02%, respectively, of the CD8 (+) T-cells were positively stained with the tetramer of Influenza NP (negative control) (Fig. 4*a*). Next, a ^{51}Cr-release assay was performed to evaluate the cytotoxic activity against the B16 cells of CD8(+) CTLs in the spleens of the mice immunized with genetically modified DCs or PBS. Although the CD8(+) CTLs in the immunized mice exhibited no cytotoxic activity against the MC38 or YAC-1 (NK-sensitive target) cells, they did express significantly higher levels of cytotoxicity against the B16 cells in the mice immunized with genetically modified BMDCs and iPSDCs expressing hgp100 than that observed in the other cells (E/T: 25, $p < 0.0001$). There were no significant differences between the genetically modified DCs expressing hgp100 in terms of the cytotoxic activity against B16 cells (E/T: 25, $p > 0.05$, Fig. 4*b*). Furthermore, to evaluate the cytotoxic activity of the draining lymph nodes cells in the mice immunized with genetically modified DCs or PBS, a ^{51}Cr-release assay was performed. Although the lymph node cells in the immunized mice exhibited no cytotoxic activity against MC38, they did express a significantly higher level of cytotoxicity against the B16 cells in the mice immunized with BMDCs-hgp100 and iPSDCs-hgp100 than that observed in the other cells (E/T: 6.25, $p < 0.05$). There were no significant differences between the genetically modified DCs expressing hgp100 in terms of the cytotoxic activity against B16 cells (E/T: 6.25, $p > 0.05$, Fig. 4*c*).

Therapeutic efficacy of genetically modified DCs in the subcutaneous tumor model

We used treatment schedules in the subcutaneous tumor model to evaluate the therapeutic efficacy of vaccination with genetically modified DCs or PBS against B16 cells. The vaccination with BMDCs-hgp100 and iPSDCs-hgp100 exhibited significantly higher therapeutic efficacy than the other types of vaccination (Day 20, $p < 0.0001$). There were no significant differences between the genetically modified DCs expressing hgp100 in terms of therapeutic efficacy (Day 20, $n = 5$, $p > 0.05$, Fig. 5).

Discussion

Our study demonstrated that iPS cells are capable of differentiating into functional DCs in four steps. In concrete terms,

Figure 4. Cytotoxic activity of CD8(+) CTLs in the mice immunized with DCs expressing hgp100. (*a*) Splenocytes were removed from the mice immunized with genetically modified DCs and cultured with rm IL-2 only. After 5 days, the cultured cells were stained with the tetramers of H-2D^b-hgp100 and H-2D^b-Influenza NP (negative control) in combination with anti-CD8 mAbs and analyzed using flow cytometry. (*b*) The cytotoxic activity of the spleen cells in the mice immunized with genetically modified DCs. The genetically modified DCs were as follows: PBS (——), BMDCs-AxCALacz (— ·), BMDCs-AxCAhgp100 (·····), iPSDCs-AxCALacZ (– –) and iPSDCs-AxCAhgp100 (—). The cytotoxic activity was examined using a ^{51}Cr -release assay. The results are shown as the mean ± SD ($n = 7$ for each group). *Significantly higher cytotoxicity against B16 cells in the mice immunized with genetically modified BMDCs and iPSDCs expressing hgp100 than that observed in the other cells (E/T: 25, $p < 0.0001$). **No significant differences compared to the genetically modified DCs expressing hgp100 (E/T: 25, $p > 0.05$). (*c*) The cytotoxic activity of the lymph node cells in the mice immunized with genetically modified DCs. The genetically modified DCs were as follows: same as above. The cytotoxic activity was examined using a ^{51}Cr-release assay. The results are shown as the mean ± SD (n=5 for each group). *Significantly higher cytotoxicity against B16 cells in the mice immunized with genetically modified BMDCs and iPSDCs expressing hgp100 than that observed in the other cells (E/T: 6.25, $p < 0.05$). **No significant differences compared to the genetically modified DCs expressing hgp100 (E/T: 6.25, $p > 0.05$).

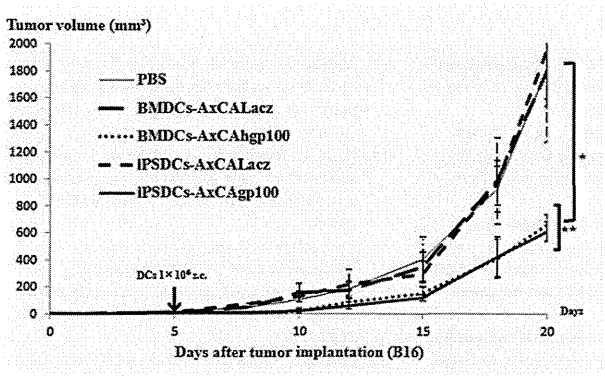

Figure 5. Therapeutic efficacy of genetically modified DCs in the subcutaneous tumor model. Tumor growth suppression in the mice immunized with genetically modified DCs in the subcutaneous tumor model ($n = 5$ for each group). The genetically modified DCs were as follows: PBS (——), BMDCs-AxCALacz (— ·), BMDCs-AxCAhgp100 (·····), iPSDCs-AxCALacZ (– –) and iPSDCs-AxCAhgp100 (—). The results are presented as the mean tumor volume ± SD of the mice that developed tumors in each group. *Significantly higher therapeutic efficacy than that observed in the other cells (Day 20, $p < 0.0001$). **No significant differences compared to the genetically modified DCs expressing hgp100 in terms of therapeutic efficacy (Day 20, $p > 0.05$).

the iPSDCs were fully matured with TNF-α and secreted adequate amounts of IL-12 and IFN-γ, as did the BMDCs. Furthermore, the iPSDCs exhibited an equal migration capacity to that of the BMDCs.

As the generation of iPS cells was first reported, several studies have evaluated organs derived from iPS cells, such as retinal pigment epithelia, platelets and gametes.[20–22] These iPS cell-derived organs demonstrate a similar capacity to naive organs. Senju *et al.*[13] examined the global gene expression profiles of BMDCs and iPSDCs using DNA microarrays. Their results indicated that the gene expression profiles of the two cell types were similar. This similarity in the gene expression likely explains the similar function observed between BMDCs and iPSDCs. Further investigation of this issue is necessary. However, our study experimentally demonstrated that iPSDCs have an equal capacity to BMDCs in terms of maturation and migration. In addition, we demonstrated that injected iPSDCs, as well as BMDCs, can migrate into the nodular paracortex and T-cell zone to interact with T-cells.[1]

The primary advantage of a gene-based vaccination strategy using DCs transduced with the entire TAA gene is that the DCs will present multiple epitopes, including unknown epitopes associated with different MHC Class I molecules, in addition to helper epitopes associated with MHC Class II molecules.[23,24] Our study demonstrated that an adenovirus vector encoding the TAA gene, hgp100, can be used to effectively transfer and express the transgene in iPSDCs. On the other hand, it is generally accepted that iPS cells exhibit high efficiency and simplicity of transgene transfection using electroporation. If iPS cells are stably transfected with the TAA gene before differentiating into DCs, almost 100% of the iPSDCs derived from these iPS cells are expected to express the TAA gene. Therefore, it is a more effective clinical application to transfect the TAA gene into iPS cells before the cells differentiate into DCs. Currently, we seek to induce the differentiation of iPSDCs from iPS cells stably expressing the TAA gene.

We showed that a single vaccination of genetically modified iPSDCs expressing the entire TAA gene elicits a potent therapeutic TAA-specific immunity that results in the suppression of tumor growth. Furthermore, in our study, the vaccination of genetically modified iPSDCs and BMDCs exhibited an equivalent antitumor effect. Several mechanisms are suggested based on the successful induction of antitumor immunity. First, iPSDCs have an equal capacity to BMDCs in terms of maturation and migration, as described above. Therefore, genetically modified iPSDCs expressing TAA at the completely matured state may express more stable MHC Class I TAA-specific peptide complexes, resulting in efficient TAA presentation in the context of costimulatory molecules. Second, iPSDCs have an equal capacity to BMDCs in terms of MHC Class I/II presentation of endogenously expressed antigens. Indeed, our results of the TAA-specific tetramer assay and TAA-specific CTL activity in both splenocytes and regional lymph node suspensions strongly suggest that CTL recognition of cross-reactive MHC Class I epitopes on the TAA molecules can be induced in mice immunized with iPSDCs. Further investigation of this issue is necessary to establish the clinical applications of this strategy.

In conclusion, vaccination with iPSDCs may solve the problems related to the defective function, number and viability of naive DCs obtained from cancer patients. We consider this strategy to be useful for clinical application as a cancer vaccine. Such therapy would include the following four steps: Step 1: culture and proliferate iPS cells generated from the patient's fibroblasts; Step 2: transduce TAA genes into the iPS cells; Step 3: induce these iPS cells into iPSDCs and Step 4: vaccinate the genetically modified iPSDCs expressing the TAA gene as a cancer therapy tailor-made for the patient. There are many issues to be overcome before this vaccination strategy can be applied in clinical practice; however, work is ongoing to meet this goal.

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ORIGINAL ARTICLE

A new prognostic score for the survival of patients with esophageal squamous cell carcinoma

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Abstract
Purpose Recent studies have shown that the modified Glasgow Prognostic Score (mGPS), which is an inflammation-based prognostic score, is useful as a prognostic index for some cancer cases. The purpose of this study was to create a prognostic scoring system for patients with esophageal squamous cell carcinoma (ESCC) that was more independent and sensitive than the mGPS.
Methods One hundred sixty-eight patients who had undergone esophagectomy for ESCC were included in the study. The new mGPS (NmGPS) was calculated based on the following cutoff values: CRP >0.75 mg/dL indicated NmGPS 1 or 2, depending on the absence or presence of hypoalbuminemia (<3.5 g/dL); and CRP ≤0.75 mg/dL indicated NmGPS 0. We also performed an analysis based on cutoff values of 0.5 and 0.25 mg/dL for CRP.
Results Only the NmGPS with a cutoff CRP value of 0.5 mg/dL was able to divide into three independent patient groups in the survival curves. In the multivariate analyses, a NmGPS (CRP cutoff; 0.5 mg/dL) of 2 was a more significant independent prognostic factor (HR 4.437, 95 % CI 2.000–9.844, $p = 0.0002$) than a mGPS of 2 (HR 2.726, 95 % CI 1.021–7.112, $p = 0.0449$).
Conclusions The new prognostic score NmGPS (CRP cutoff; 0.5 mg/dL) was more independent and sensitive than the mGPS for patients with ESCC.

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Keywords Esophageal squamous cell carcinoma · Inflammation-based prognostic score · Glasgow Prognostic Score (GPS)

Abbreviations

GPS	Glasgow Prognostic Score
mGPS	Modified Glasgow Prognostic Score
NmGPS	New modified Glasgow Prognostic Score
CRP	C-reactive protein
ESCC	Esophageal squamous cell carcinoma
AIC	Akaike information criterion
HR	Hazard ratio
CI	Confidence interval
SIR	Systemic inflammatory response
IL-6	Interleukin-6

Introduction

Esophageal cancer is the eighth-most common cancer worldwide and the sixth-most common cause of cancer death [1]. The predominant histological subtype of esophageal cancer is squamous cell carcinoma (SCC), which comprises about 80 % of all esophageal cancers worldwide [2]. In Asian countries, especially Japan, China and Iran, about 90 % of esophageal cancers are SCC [3]. On the other hand, in the United States and other Western countries, about 50 % of esophageal cancers are adenocarcinomas [4]. Because there are important biological differences between esophageal cancers in Asian and Western countries, a prognostic model that takes into account the predominance of SCC in Asian countries is necessary for deciding on a postoperative strategy that will prolong survival. Markers predicting the malignant potential of tumors and the prognosis of patients with esophageal

squamous cell carcinoma (ESCC) would be extremely useful.

Previous reports have shown that systemic inflammation, indicated by an elevated level of serum C-reactive protein (CRP), strongly influenced the prognosis of patients with gastrointestinal carcinomas, including gastro-esophageal cancer [5] and colorectal cancer [6]. Moreover, the combination of an elevated serum CRP level and hypoalbuminemia is considered to be an important prognostic factor [7]. Indeed, the Glasgow prognostic score (GPS), which is based on the serum CRP level and hypoalbuminemia, has been demonstrated to be an indicator for the prognosis of patients with neoplasms of the digestive tract, including colorectal cancer [8], gastric cancer [9] and pancreatic cancer [10]. The GPS seemed to be a simple prognostic indicator for human tumors and was calculated easily as follows: a score of 0 for normal C-reactive protein and albumin levels, 1 for either an abnormal C-reactive protein or abnormal albumin level and 2 for abnormal C-reactive protein and abnormal albumin levels. Because hypoalbuminemia alone was not associated with reduced survival [11], recent reports have demonstrated the superior usefulness of the modified GPS (mGPS) for prognostication of the mortality in patients with colorectal cancer [12], pancreatic cancer [13] or gastric cancer [14].

However, some of the studies using the GPS or mGPS have had various problems. For example, since the number of patients with GPS 1 or 2 (mGPS 1 or 2) was significantly smaller than the number of patients with a 0 score [15], the GPS or mGPS may have an obvious bias. Moreover, there was no significant difference found between the survival curves of patients with GPS 1 and those with GPS 0 or 2 [9, 16]. In addition, neither the GPS nor mGPS was able to be separated into three independent patient groups with each GPS (mGPS) according to the survival curves. Therefore, the GPS and mGPS may be considered insufficient for prognostication. A new version of the GPS is therefore necessary to improve the prognostication. We herein investigated three new CRP cutoff values to establish a new mGPS (NmGPS) for esophageal cancer, especially SCC. Then, we compared the mGPS with the NmGPS with respect to the clinicopathological features and prognostic significance. The purpose of this study was to create a prognostic scoring system for patients with potentially resectable ESCC that is more sensitive than the mGPS.

Materials and methods

Patients, blood samples and laboratory measurements

One hundred sixty-eight patients with esophageal cancer who had been treated by esophagectomy and lymph node dissection from January 2003 through December 2008 at Wakayama Medical University Hospital (WMUH) were evaluated. The patients who had other malignant tumors, who had other inflammatory diseases causing elevated levels of serum CRP, who died immediately after surgery (within 30 days) or who died because of non-cancer-related causes were excluded from this study. Patients with R1 or R2 status were included to evaluate aggressive tumor biology. All blood samples were collected and tested for the serum CRP and albumin levels just before surgery. The scores were evaluated as follows: First, we assessed the mGPS, as previously described by several groups [12–14]. Patients with an elevated level of CRP (>1.0 mg/dL) were assigned a mGPS of 1 or 2, depending on the absence or presence of hypoalbuminemia (<3.5 g/dL), and patients who did not have an elevated level of CRP (≤1.0 mg/dL) were assigned a mGPS of 0. We assessed the values of our new version of the mGPS (NmGPS) using three CRP cutoff values: 0.75, 0.5 and 0.25 mg/dL. Initially, patients with an elevated level of CRP (>0.75 mg/dL) were assigned a NmGPS (CRP cutoff; 0.75 mg/dL) of 1 or 2, depending on the absence or presence of hypoalbuminemia (<3.5 g/dL). Patients without an elevated level of CRP (≤0.75 mg/dL) were assigned a NmGPS (CRP cutoff; 0.75 mg/dL) of 0. Alternate NmGPS values were assigned based on CRP cutoffs of 0.5 mg/dL and 0.25 mg/dL. We selected the most appropriate new mGPS from these three models and then compared the mGPS with the NmGPS with respect to their relationships with the clinicopathological features and their prognostic significance. The primary objective was to decide which CRP value was the most specific for cancer-specific survival. This study was approved by the Ethical Committee on Clinical Investigation of WMUH. Informed consent was obtained from all participating patients preoperatively.

The pathological classification of the primary tumor, the degree of lymph node involvement and the presence of organ metastasis were determined according to the TNM classification system (7th edition of the cancer staging manual of the American Joint Committee on Cancer [17]).

Surgical procedure

All patients underwent radical esophagectomy with a two- or three-field lymph node dissection via a cervicothoracoabdominal approach. A gastric conduit through the retrosternal rout or through the posterior mediastinum was usually used to reconstruct the anastomosis with the cervical esophagus. However, in patients, who had undergone a prior gastrectomy or who had concomitant gastric cancer, the right ileocolon was used.

Neoadjuvant therapy and adjuvant therapy

Beginning in 2005, we administered neoadjuvant chemotherapy for patients with clinical lymph node metastases,

and neoadjuvant chemoradiotherapy for patients with clinical T4 lesions. We performed esophagectomy about 1 month after the end of neoadjuvant therapy, when the inflammatory response due to the neoadjuvant therapy would have disappeared. Nineteen patients received neoadjuvant therapy. Among them, 12 received chemotherapy consisting of 5-fluorouracil/cisplatin, and seven received radiotherapy with chemotherapy. Seventy-seven patients received postoperative adjuvant therapy. Among them, 74 with pathological lymph node metastases received chemotherapy consisting of 5-fluorouracil/cisplatin or 5-fluorouracil/cisplatin/docetaxel, and three with residual tumors received radiotherapy with chemotherapy.

Patient follow-up

All patients were followed up at regular intervals after discharge from our hospital. This included blood tests for tumor markers, such as carcinoembryonic antigen (CEA), SCC and cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) every 3 months, and a follow-up CT scan and an endoscopic examination every 6 months. As of the writing of this manuscript, the median follow-up time for surviving patients was 39 (range 5–99) months. None of the patients were lost to follow-up.

Statistical analysis

The data are presented as prevalences or medians. The Chi-square test was used to test for correlations between the clinicopathological parameters and prognostic scores. The cancer-specific survival curves were estimated by the Kaplan–Meier method, and the prognostic factors were assessed by the log-rank test and a Cox proportional hazards model for the univariate and multivariate analyses. The results are presented as hazard ratios (HRs) with 95 % confidence intervals (CIs). Moreover, we used the Akaike information criterion (AIC) to identify the best statistical model [18]. The AIC was defined as: AIC = -2 log maximum likelihood + 2 $\times$ (the number of parameters in the model). A smaller AIC value indicates a more desirable model for predicting the outcome. The statistical analyses were performed with the SPSS Version 21.0 software program (SPSS Inc., Chicago, IL, USA). A $p < 0.05$ was considered to be statistically significant.

Results

The study included 168 patients with histological subtype ESCC. The clinicopathological features are summarized in Table 1. The study population included 135 males and 33 females aged 47–85 years (median age 67 years).

The 3-year cancer-specific survival rates of the patients with mGPS 0, 1 and 2 were 81.7, 42.8 and 31.3 %, respectively. There were statistically significant differences between the survival of patients with mGPS 0 and 1 ($p = 0.0002$), and between patients with mGPS 0 and 2 ($p < 0.0001$). There was no significant difference between the survival rates of patients with mGPS 1 and 2 ($p = 0.1860$) (Fig. 1a). The 3-year cancer-specific survival rates of patients with NmGPS (CRP cutoff; 0.75 mg/dL) 0, 1 and 2 were 82.3, 43.6 and 28.8 %, respectively. There were statistically significant differences between the survival rates of patients with NmGPS (CRP cutoff; 0.75 mg/dL) 0 and 1 ($p = 0.0001$), and between patients with NmGPS (CRP cutoff; 0.75 mg/dL) 0 and 2 ($p < 0.0001$). There was no significant difference between the survival rates of patients with NmGPS (CRP cutoff; 0.75 mg/dL) 1 and 2 ($p = 0.1171$) (Fig. 1b). The 3-year cancer-specific survival rates of patients with NmGPS (CRP cutoff; 0.5 mg/dL) 0, 1 and 2 were 86.5, 51.0 and 27.8 %, respectively. There were statistically significant differences between the survival rates of patients with NmGPS (CRP cutoff; 0.5 mg/dL) 0 and 1 ($p < 0.0001$), between patients with NmGPS (CRP cutoff; 0.5 mg/dL) 0 and 2 ($p < 0.0001$) and between patients with NmGPS (CRP

Table 1 Clinicopathological features of the patients

Sex ratio, male/female	135/33
Median age (range), years	67 (47–85)
mGPS 0/1/2, *n*	137/19/12
NmGPS (CRP cutoff; 0.75 mg/dL) 0/1/2, *n*	135/20/13
NmGPS (CRP cutoff; 0.5 mg/dL) 0/1/2, *n*	121/26/21
NmGPS (CRP cutoff; 0.25 mg/dL) 0/1/2, *n*	88/56/24
Histological type, *n*	
Differentiated/undifferentiated	117/51
Lymphatic invasion 0/1, 2, 3	66/102
Venous invasion 0/1, 2, 3	86/82
Median maximum tumor size (range), mm	40 (5–100)
Two-/three-field lymph node dissection, *n*	62/106
Depth of tumor invasion T0/1/2/3/4, *n*	5/59/22/74/8
Lymph node metastasis N0/1/2/3, *n*	68/25/32/43
Distant metastasis M0/1, *n*	166/2
TMN stage 0/IA/IB/IIA/IIB/IIIA/IIIB/IIIC/IV, *n*	6/31/8/18/13/25/15/50/2
Residual tumor R0/1, 2, *n*	159/9
Neoadjuvant therapy yes/no, *n*	19/149
Chemotherapy/CRT, *n*	12/7
Adjuvant therapy yes/no, *n*	77/91
Chemotherapy/CRT, *n*	74/3

mGPS modified Glasgow Prognostic Score, *NmGPS* new modified Glasgow Prognostic Score, *T* tumor, *N* node, *M* metastasis, *CRT* chemoradiotherapy

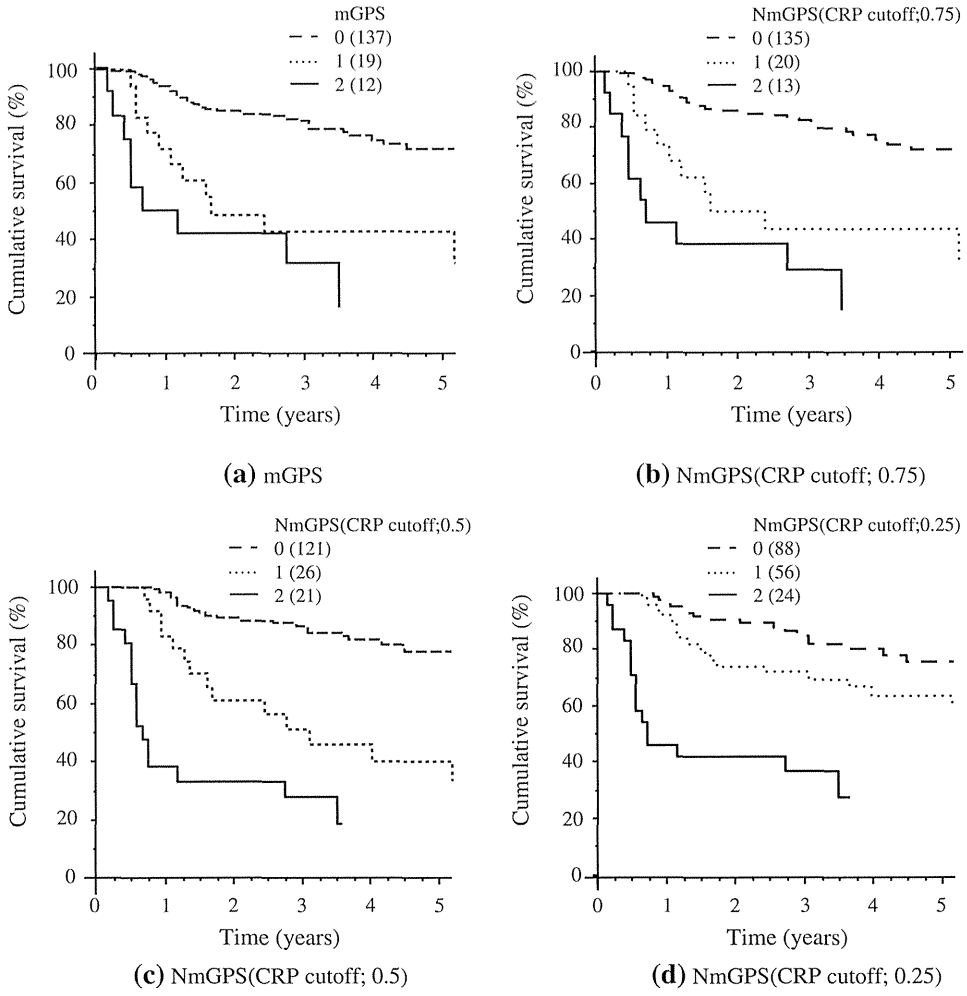

Fig. 1 **a** The cancer-specific survival curves based on the modified Glasgow Prognostic Score (mGPS). The 3-year cancer-specific survival rates of patients with a mGPS of 0 (*dashed line*), 1 (*dotted line*) and 2 (*solid line*) were 81.7, 42.8 and 31.3 %, respectively. There were statistically significant differences between the survival rates of patients with a mGPS of 0 and 1 ($p = 0.0002$), and between patients with a mGPS of 0 and 2 ($p < 0.0001$). There was no significant difference between the survival rates of patients with a mGPS of 1 and 2 ($p = 0.1860$). **b** The cancer-specific survival curves based on the new modified Glasgow Prognostic Score (NmGPS [CRP cutoff; 0.75 mg/dL]). The 3-year cancer-specific survival rates of patients with a NmGPS (CRP cutoff; 0.75 mg/dL) of 0 (*dashed line*), 1 (*dotted line*) and 2 (*solid line*) were 82.3, 43.6 and 28.8 %, respectively. There were statistically significant differences between the survival rates of patients with a NmGPS (CRP cutoff; 0.75 mg/dL) of 0 and 1 ($p = 0.0001$), and between patients with a NmGPS (CRP cutoff; 0.75 mg/dL) of 0 and 2 ($p < 0.0001$). There was no significant difference between the survival rates of patients with a NmGPS (CRP cutoff; 0.75 mg/dL) of 1 and 2 ($p = 0.1171$). **c** The cancer-specific survival curves based on the new modified Glasgow Prognostic Score (NmGPS [CRP cutoff; 0.5 mg/dL]). The 3-year cancer-specific survival rates of patients with a NmGPS (CRP cutoff; 0.5 mg/dL) of 0 (*dashed line*), 1 (*dotted line*) and 2 (*solid line*) were 86.5, 51.0 and 27.8 %, respectively. There were statistically significant differences between the survival rate of patients with a NmGPS (CRP cutoff; 0.5 mg/dL) of 0 and 1 ($p < 0.0001$), of 0 and 2 ($p < 0.0001$), and between patients with a NmGPS (CRP cutoff; 0.5 mg/dL) of 1 and 2 ($p = 0.0099$). **d** The cancer-specific survival curves based on the new modified Glasgow Prognostic Score (NmGPS [CRP cutoff; 0.25 mg/dL]). The 3-year cancer-specific survival rates of patients with a NmGPS (CRP cutoff; 0.25 mg/dL) of 0 (*dashed line*), 1 (*dotted line*) and 2 (*solid line*) were 85.1, 71.9 and 36.5 %, respectively. There were statistically significant differences between the survival rates of patients with a NmGPS (CRP cutoff; 0.25 mg/dL) of 0 and 2 ($p < 0.0001$), and between patients with a NmGPS (CRP cutoff; 0.25 mg/dL) of 1 and 2 ($p < 0.0001$), but not between those with a NmGPS (CRP cutoff; 0.25 mg/dL) of 0 and 1 ($p = 0.0685$)

cutoff; 0.5 mg/dL) 1 and 2 ($p = 0.0099$) (Fig. 1c). The 3-year cancer-specific survival rates of patients with NmGPS (CRP cutoff; 0.25 mg/dL) 0, 1 and 2 were 85.1, 71.9 and 36.5 %, respectively. There were statistically significant differences between the survival of patients with NmGPS (CRP cutoff; 0.25 mg/dL) 0 and 2 ($p < 0.0001$) and between patients with NmGPS (CRP cutoff; 0.25 mg/dL) 1 and 2 ($p < 0.0001$), but not between patients with NmGPS (CRP cutoff; 0.25 mg/dL) 0 and 1 ($p = 0.0685$) (Fig. 1d). Only NmGPS (CRP cutoff; 0.5 mg/dL) was able to divide into three independent patient groups in the survival curves.

Table 2 The Akaike information criterion (AIC) for the prognostic scoring systems

Prognostic score	AIC
mGPS	1059.0
NmGPS (CRP cutoff; 0.75 mg/dL)	1055.8
NmGPS (CRP cutoff; 0.5 mg/dL)	1044.9
NmGPS (CRP cutoff; 0.25 mg/dL)	1054.1

mGPS modified Glasgow Prognostic Score, *NmGPS* new modified Glasgow Prognostic Score

The AIC of the mGPS, NmGPS (CRP cutoff; 0.75 mg/dL), NmGPS (CRP cutoff; 0.5 mg/dL) and NmGPS (CRP cutoff; 0.25 mg/dL) were 1059.0, 1055.8, 1044.9 and 1054.1, respectively (Table 2). The NmGPS (CRP cutoff; 0.5 mg/dL) showed a smaller AIC than the mGPS and other NmGPS, so we selected the NmGPS (CRP cutoff; 0.5 mg/dL) as the optimal prognostic score and compared the mGPS with the NmGPS (CRP cutoff; 0.5 mg/dL) with respect to the clinicopathological features and prognostic

Table 3 The relationships between the mGPS and the clinicopathological features

Variable	mGPS 0 (n = 137)	mGPS 1 (n = 19)	mGPS 2 (n = 12)	p
Age, years				0.6128
≤65	66 (48.2 %)	7 (36.8 %)	5 (41.7 %)	
>65	71 (51.8 %)	12 (63.2 %)	7 (58.3 %)	
Sex				0.5084
Male	108 (78.8 %)	16 (84.2 %)	11 (91.7 %)	
Female	29 (21.2 %)	3 (15.8 %)	1 (8.3 %)	
Depth of tumor invasion				<0.0001
T0/1/2	81 (59.1 %)	3 (15.8 %)	2 (16.7 %)	
T3/4	56 (40.9 %)	16 (84.2 %)	10 (83.3 %)	
Lymph node metastasis				0.0101
N0	61 (44.5 %)	7 (36.8 %)	0 (0 %)	
N1/2/3	76 (55.5 %)	12 (63.2 %)	12 (100 %)	
Distant metastasis				<0.0001
M0	137(100 %)	19 (100 %)	10 (83.3 %)	
M1	0	0	2 (16.7 %)	
Histological type				0.2552
Differentiated	99 (72.3 %)	14 (73.7 %)	6 (50.0 %)	
Undifferentiated	38 (27.7 %)	5 (26.3 %)	6 (50.0 %)	
Lymphatic invasion				0.4047
0	57 (41.6 %)	6 (31.6 %)	3 (25.0 %)	
1, 2, 3	80 (58.4 %)	13 (68.4 %)	9 (75.0 %)	
Venous invasion				0.0043
0	77 (56.2 %)	3 (15.8 %)	6 (50.0 %)	
1, 2, 3	60 (43.8 %)	16 (84.2 %)	6 (50.0 %)	
Maximum tumor size, mm				0.0004
≤40	80 (58.4 %)	4 (21.1 %)	2 (16.7 %)	
>40	57 (41.6 %)	15 (78.9 %)	10 (83.3 %)	
Lymph node dissection				0.2819
Two fields	47 (34.3 %)	10 (52.6 %)	5 (41.7 %)	
Three fields	90 (65.7 %)	9 (47.4 %)	7 (58.3 %)	
Neoadjuvant therapy				0.0436
Yes	13 (9.5 %)	2 (10.5 %)	4 (33.3 %)	
No	124 (90.5 %)	17 (89.5 %)	8 (66.7 %)	
Adjuvant therapy				0.6440
Yes	62 (45.3 %)	8 (42.1 %)	7 (58.3 %)	
No	75 (54.7 %)	11 (57.9 %)	5 (41.7 %)	
TNM stage				0.0013
0, I, II	70 (51.1 %)	6 (31.6 %)	0	
III, IV	67 (48.9 %)	13 (68.4 %)	12 (100 %)	
Residual tumor				<0.0001
R0	136 (99.3 %)	16 (84.2 %)	7 (58.3 %)	
R1/2	1 (0.7 %)	3 (15.8 %)	5 (41.7 %)	

mGPS modified Glasgow Prognostic Score, *T* tumor, *N* node, *M* metastasis, *R* residual tumor

Table 4 The univariate prognostic factors for esophageal cancer (including the mGPS)

Variable	*p*	HR	95 % CI
Sex (male)	0.4597	1.312	0.639–2.694
Age (>65 years)	0.1343	1.550	0.873–2.751
mGPS (2)	<0.0001	5.664	2.717–11.809
Depth of tumor invasion (T3, 4)	<0.0001	3.801	2.057–7.025
Lymph node metastasis			
N1	0.0215	3.064	1.179–7.962
N2	0.0165	3.476	1.256–9.625
N3	<0.0001	10.200	4.575–22.739
Distant metastasis (M1)	<0.0001	3.745	2.165–6.476
Histological type (undifferentiated)	0.4363	0.765	0.390–1.501
Lymphatic invasion (1, 2, 3)	0.0047	2.541	1.332–4.850
Venous invasion (1, 2, 3)	0.0021	2.453	1.383–4.351
Tumor size (>40 mm)	0.0013	2.750	1.484–5.098
Lymph node dissection (three fields)	0.6601	1.142	0.632–2.064
Neoadjuvant therapy	0.5469	1.302	0.552–3.069
Adjuvant therapy	0.0020	2.468	1.392–4.374
Residual tumor (R1, 2)	<0.0001	17.248	7.826–38.016

mGPS modified Glasgow Prognostic Score, *HR* hazard ratio, *CI* confidence interval, *T* tumor, *N* node, *M* metastasis

Table 5 The multivariate prognostic factors for esophageal cancer (including the mGPS)

Variable	*p*	HR	95 % CI
mGPS (2)	0.0449	2.726	1.021–7.112
Depth of tumor invasion (T3, 4)	0.1884	1.718	0.767–3.848
Lymph node metastasis			
N1	0.1074	2.620	0.811–8.464
N2	0.0625	2.729	0.949–7.847
N3	0.0178	3.964	1.269–12.383
Distant metastasis (M1)	0.2256	1.569	0.757–3.250
Lymphatic invasion (1, 2, 3)	0.3861	0.711	0.329–1.538
Venous invasion (1, 2, 3)	0.0464	2.274	1.016–5.912
Tumor size (>40 mm)	0.5015	1.293	0.611–2.737
Adjuvant therapy	0.2073	1.589	0.773–3.266
Residual tumor (R1, 2)	0.0152	3.605	1.280–10.149

mGPS modified Glasgow Prognostic Score, *HR* hazard ratio, *CI* confidence interval, *T* tumor, *N* node, *M* metastasis

significance. The relationships between mGPS and clinicopathological features are summarized in Table 3.

Statistically significant associations were detected between the prognostics scores and the tumor depth ($p < 0.0001$), lymph node metastasis ($p = 0.0101$), distant metastasis ($p < 0.0001$), venous invasion ($p = 0.0043$), maximum tumor size ($p = 0.0004$), neoadjuvant therapy ($p = 0.0436$), advanced stage ($p = 0.0013$) and residual tumor R1/2 ($p < 0.0001$). The univariate analysis demonstrated that a mGPS of 2 ($p < 0.0001$), the depth of tumor invasion ($p < 0.0001$), lymph node metastasis (N1) ($p = 0.0215$), N2 ($p = 0.0165$), N3 ($p < 0.0001$), distant metastasis ($p < 0.0001$), lymphatic invasion ($p = 0.0047$), venous invasion ($p = 0.0021$), the maximum tumor size ($p = 0.0013$), neoadjuvant therapy ($p = 0.0020$) and R1/2 status ($p < 0.0001$) were associated with a worse prognosis (Table 4). The multivariate analysis demonstrated that a mGPS of 2 ($p = 0.0449$), lymph node metastasis (N3) ($p = 0.0178$), venous invasion ($p = 0.0464$) and R1/2 tumors ($p = 0.0152$) were independently associated with a worse prognosis (Table 5).

The relationships between the NmGPS (CRP cutoff; 0.5 mg/dL) and clinicopathological features are summarized in Table 6. Similar statistically significant differences were detected regarding the depth of tumor invasion ($p < 0.0001$), lymph node metastasis ($p = 0.0008$), distant metastasis ($p = 0.0008$), venous invasion ($p = 0.0015$), maximum tumor size ($p < 0.0001$), neoadjuvant therapy ($p = 0.0007$), adjuvant therapy ($p = 0.0166$), advanced stage ($p < 0.0001$) and R1/2 status ($p < 0.0001$). In the univariate analysis, a NmGPS (CRP cutoff; 0.5 mg/dL) of 2 was associated with a worse prognosis ($p < 0.0001$) (Table 7). The multivariate analysis demonstrated that a NmGPS (CRP cutoff; 0.5 mg/dL) of 2 ($p = 0.0002$), lymph node metastasis (N3) ($p = 0.0201$), venous invasion ($p = 0.0190$) and R1/2 status ($p = 0.0209$) were independently associated with a worse prognosis (Table 8).

Discussion

We evaluated the associations between the serum CRP and serum albumin levels with the clinicopathological features and cancer-specific survival of patients with ESCC. Moreover, we established a prognostic scoring system more sensitive than the mGPS, with a lower CRP cutoff value. This study is the first to evaluate a new, optimal mGPS, and to compare it with the mGPS for patients with ESCC. In fact, the NmGPS (CRP cutoff; 0.5 mg/dL) could indicate more aggressive tumor biology, in terms of the depth of tumor invasion, presence of lymph node metastasis, distant metastasis, venous invasion, the maximum tumor size, advanced stage and R1/2 status. The number of patients with a mGPS of 2 was very small (7.1 %), in contrast to the number of patients with a mGPS 0 or 1. Moreover, there were no significant differences between the survival curves of patients with a mGPS of 1 and 2. Therefore, the mGPS was unable to be separated into three independent patient groups. On the other hand, when patients were classified based on the NmGPS (CRP cutoff; 0.5 mg/dL), there were significant differences between

Table 6 The relationships between the NmGPS (CRP cutoff; 0.5 mg/dL) and the clinicopathological features

Variable	NmGPS (0.5) 0 (n = 121)	NmGPS (0.5) 1 (n = 26)	NmGPS (0.5) 2 (n = 21)	p
Age, years				0.7471
≤65	56 (46.3 %)	10 (38.5 %)	10 (47.6 %)	
>65	65 (53.7 %)	16 (61.5 %)	11 (52.4 %)	
Sex				0.1744
Male	96 (79.3 %)	24 (92.3 %)	15 (71.4 %)	
Female	25 (20.7 %)	2 (7.7 %)	6 (28.6 %)	
Depth of tumor invasion				<0.0001
T0/1/2	78 (64.5 %)	5 (19.2 %)	3 (14.3 %)	
T3/4	43 (35.5 %)	21 (80.8 %)	18 (85.7 %)	
Lymph node metastasis				0.0008
N0	58 (47.9 %)	9 (34.6 %)	1 (4.8 %)	
N1/2/3	63 (52.1 %)	17 (65.4 %)	20 (95.2 %)	
Distant metastasis				0.0008
M0	121 (100 %)	26 (100 %)	19 (90.5 %)	
M1	0	0	2 (9.5 %)	
Histological type				0.1994
Differentiated	87 (71.9 %)	21 (80.8 %)	12 (57.1 %)	
Undifferentiated	34 (28.1 %)	5 (19.2 %)	9 (42.9 %)	
Lymphatic invasion				0.1399
0	53 (43.8 %)	8 (30.8 %)	5 (23.8 %)	
1, 2, 3	68 (56.2 %)	18 (69.2 %)	16 (76.2 %)	
Venous invasion				0.0015
0	72 (59.5 %)	6 (23.1 %)	8 (38.1 %)	
1, 2, 3	49 (40.5 %)	20 (76.9 %)	13 (61.9 %)	
Maximum tumor size, mm				<0.0001
≤40	79 (65.3 %)	6 (23.1 %)	4 (19.0 %)	
>40	42 (34.7 %)	20 (76.9 %)	17 (81.0 %)	
Lymph node dissection				0.4501
Two fields	44 (36.4 %)	12 (46.2 %)	6 (28.6 %)	
Three fields	77 (63.6 %)	14 (53.8 %)	15 (71.4 %)	
Neoadjuvant therapy				0.0007
Yes	11 (9.1 %)	2 (7.7 %)	8 (38.1 %)	
No	110 (90.9 %)	24 (92.3 %)	13 (61.9 %)	
Adjuvant therapy				0.0166
Yes	52 (43.0 %)	11 (42.3 %)	16 (76.2 %)	
No	69 (57.0 %)	15 (57.7 %)	5 (23.8 %)	
TNM stage				<0.0001
0, I, II	68 (56.2 %)	8 (30.8 %)	0	
III, IV	53 (43.8 %)	18 (69.2 %)	21 (100 %)	
Residual tumor				<0.0001
R0	121 (100 %)	23 (88.5 %)	15 (71.4 %)	
R1/2	0 (0 %)	3 (11.5 %)	6 (28.6 %)	

NmGPS new modified Glasgow Prognostic Score, *T* tumor, *N* node, *M* metastasis, *R* residual tumor

groups separated by one point (NmGPS [CRP cutoff; 0.5] 0 vs 1: $p < 0.0001$, NmGPS [CRP cutoff; 0.5 mg/dL] 1 vs 2: $p = 0.0099$). In the multivariate analysis of cancer-specific survival, a NmGPS (CRP cutoff; 0.5 mg/dL) of 2 was found to be a more independent prognostic indicator of a worse prognosis than a mGPS of 2. Moreover, we evaluated the quality of the prognostic scoring system using the AIC. The results of that evaluation suggested that the system using the NmGPS (CRP cutoff; 0.5 mg/dL) had higher quality than the mGPS and other NmGPS. These findings demonstrate that the NmGPS (CRP cutoff; 0.5 mg/dL) is more sensitive than the mGPS in patients with ESCC.

It is known that Asian countries, especially Japan, Korea and China, have the highest rates of esophageal cancer in the world [3], and people in Japan, Korea and China may be closely related in terms of genetics [19]. Shah et al. [20] reported that the mean CRP value of East Asians was less

Table 7 The univariate prognostic factors for esophageal cancer (including the NmGPS [CRP cutoff; 0.5 mg/dL])

Variable	*p*	HR	95 % CI
Sex (male)	0.4597	1.312	0.639–2.694
Age (>65 years)	0.1343	1.550	0.873–2.751
NmGPS (CRP cutoff; 0.5 mg/dL) (2)	<0.0001	7.807	4.215–14.461
Depth of tumor invasion (T3, 4)	<0.0001	3.801	2.057–7.025
Lymph node metastasis			
N1	0.0215	3.064	1.179–7.962
N2	0.0165	3.476	1.256–9.625
N3	<0.0001	10.200	4.575–22.739
Distant metastasis (M1)	<0.0001	3.745	2.165–6.476
Histological type (undifferentiated)	0.4363	0.765	0.390–1.501
Lymphatic invasion (1, 2, 3)	0.0047	2.541	1.332–4.850
Venous invasion (1, 2, 3)	0.0021	2.453	1.383–4.351
Tumor size (>40 mm)	0.0013	2.750	1.484–5.098
Lymph node dissection (three fields)	0.6601	1.142	0.632–2.064
Neoadjuvant therapy	0.5469	1.302	0.552–3.069
Adjuvant therapy	0.0020	2.468	1.392–4.374
Residual tumor (R1, 2)	<0.0001	17.248	7.826–38.016

NmGPS new modified Glasgow Prognostic Score, *HR* hazard ratio, *CI* confidence interval, *T* tumor, *N* node, *M* metastasis

Table 8 The multivariate prognostic factors for esophageal cancer (including the NmGPS [CRP cutoff; 0.5 mg/dL])

Variable	*p*	HR	95 % CI
NmGPS (CRP cutoff; 0.5 mg/dL) (2)	0.0002	4.437	2.000–9.844
Depth of tumor invasion (T3, 4)	0.2794	1.583	0.688–3.642
Lymph node metastasis			
N1	0.1379	2.236	0.772–6.476
N2	0.1119	2.553	0.804–8.108
N3	0.0201	3.731	1.229–11.323
Distant metastasis (M1)	0.3020	1.470	0.707–3.054
Lymphatic invasion (1, 2, 3)	0.3928	0.710	0.323–1.558
Venous invasion (1, 2, 3)	0.0190	2.286	1.145–4.563
Tumor size (>40 mm)	0.5685	1.251	0.579–2.702
Adjuvant therapy	0.0949	1.807	0.902–3.618
Residual tumor (R1, 2)	0.0209	3.230	1.194–8.737

NmGPS new modified Glasgow Prognostic Score, *HR* hazard ratio, *CI* confidence interval, *T* tumor, *N* node, *M* metastasis

than half the mean CRP value of people in other countries. Regarding the cause of the low CRP in East Asia, it was suggested that the haplotype map (HapMap) frequencies of CRP polymorphisms known to be associated with the CRP concentration might differ by ancestry; but for the most part, the difference in CRP is still unexplained [20]. Therefore, it could be that the low CRP cutoff value, we identified reflects the low mean CRP value of East Asians.

The mechanism responsible for the association between a systemic inflammatory response (SIR) and a poor outcome in patients with advanced cancer is not well understood. However, there is increasing evidence that there is a relationship between SIR and cancer survival. Cancer cells might influence the tumor microenvironment through the upregulation of inflammatory pathways by producing proinflammatory mediators, such as cytokines, chemokines, cyclooxygenase-2 (COX-2), prostaglandins, inducible nitric oxide synthase and nitric oxide [21]. These proinflammatory mediators markedly promote tumor progression, invasion and metastasis [21]. Interleukin-6 (IL-6) is a proinflammatory cytokine associated with angiogenesis, and it induces both the development and progression of cancer [22]. CRP is produced by hepatocytes in response to inflammatory cytokines, particularly interleukin-6, which is present in the tumor microenvironment [23]. Because the SIR is also associated with lymphocytopenia and an impaired T-lymphocytic response within the tumor microenvironment, it reflects compromised cell-mediated immunity [23].

Hypoalbuminemia often develops secondary to an ongoing SIR [24]. In addition, the occurrence of a SIR and the associated nutritional decline may influence the

tolerance of and compliance with active treatment [11]. Therefore, the combination of an elevated serum CRP level and hypoalbuminemia reflects both SIR and the progressive nutritional decline of the patient with advanced cancer, and can predict the malignant potential of the tumor and a worse prognosis of cancer patients.

CRP and albumin are routinely evaluated parameters. The GPS is simpler and cheaper than other techniques such as computed tomography, magnetic resonance imaging and positron emission tomography. Therefore, because we can easily predict the prognosis of cancer patients using the NmGPS (CRP cutoff; 0.5 mg/dL), we can take appropriate measures to care for postoperative patients to improve their survival.

In conclusion, we developed a simple and sensitive prognostic scoring system for patients with esophageal squamous cell carcinoma based on the GPS. This scoring system may also be useful for predicting the prognosis of patients with other carcinomas.

Conflict of interest M. Nakamura and the co-authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

Atrial fibrillation after esophageal cancer surgery: an analysis of 207 consecutive patients

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Abstract
Purpose The aim of this study was to identify perioperative risk factors that are associated with postoperative atrial fibrillation (AF) and the outcomes of different pharmacological interventions in esophageal cancer patients who underwent transthoracic esophagectomy.
Methods This study included 207 patients who underwent a transthoracic esophagectomy for esophageal cancer resection by a single surgeon from January 1, 2004, through December 31, 2010.
Results Postoperative AF occurred in 19 patients (9.2 %), all of whom received antiarrhythmic drug therapy at the early stage. Antiarrhythmic treatment was effective in 12 cases (63.2 %). In this study, landiolol hydrochloride, an ultrashort-acting β_1-selective β-blocker, was the first-line therapy for postoperative AF. A multivariate logistic regression analysis showed that postoperative AF was significantly associated with the use of an ileo-colon for reconstruction after esophagectomy ($P = 0.0023$, odds ratios [OR] = 13.6) and with the presence of tachycardia with a heart rate of >100 bpm on postoperative day (POD) 1 ($P = 0.0004$, OR = 18.4).
Conclusions Postoperative AF is associated with the use of a colon conduit for reconstruction after esophagectomy and with tachycardia with a heart rate >100 bpm on POD 1. Identifying patients at high risk for postoperative AF will allow for more direct application of pharmacological methods of prophylaxis.

Keywords Esophageal cancer · Atrial fibrillation · Esophagectomy

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Introduction

A transthoracic esophagectomy for the resection of esophageal carcinoma is associated with a high incidence of complications, including postoperative pneumonia, anastomotic leakage and cardiac events. Postoperative atrial fibrillation (AF) is a common arrhythmia after esophagectomy (10–60 %) and is associated with increased morbidity and mortality rates [1–6]. Considerable progress has been made toward decreasing surgical complications, such as anastomotic leakage, because of the standardization of surgical techniques [7]. However, the continued high rate of postoperative AF may be related to the use of extensive lymphadenectomy [8, 9]. Several studies have reported on the use of pharmacological therapies, such as diltiazem, amiodarone and landiolol, to prevent AF after general thoracic surgery, but their effectiveness remains controversial [10–13]. Therefore, the identification of high-risk populations will allow for targeted use, and will resolve questions about the efficacy of the drugs, potentially leading to successful prevention of AF.

Previous studies identified several risk factors predicting the development of AF after esophagectomy, including advanced age, male sex, a history of cardiac disease and a history of chronic obstructive pulmonary disease (COPD) [2, 4, 14]. However, these studies examined only small numbers of patients with esophageal cancer, and the risk

factors are consequently not clearly understood. Furthermore, these studies included patients who had various surgical procedures, such as the Lewis Tanner operation, transhiatal approach and three-phase lymph node dissection. We routinely perform right transthoracic esophageal resection with two-field (the mediastinum and the abdomen) or three-field (plus bilateral cervix) lymph node dissection and anastomosis with the cervical esophagus in the cervical wound for patients with thoracic esophageal cancer. For these reasons, our study was conducted to identify perioperative risk factors that are associated with postoperative AF, and to assess the outcomes of different pharmacological interventions in esophageal cancer patients who underwent transthoracic esophagectomy.

Patients and methods

Between January 1, 2004, and December 31, 2010, a total of 232 consecutive patients underwent surgery for thoracic esophageal carcinoma at Wakayama Medical University Hospital. This study included 207 patients who underwent transthoracic esophagectomy for esophageal cancer resection by a single surgeon. The patients who underwent esophagectomy with an additional laryngectomy ($n = 2$) or pneumonectomy ($n = 2$) were excluded. Two patients who underwent a nonresective operation (bypass surgery, 1 patient; diagnostic thoracotomy, 1 patient) were also excluded. In addition, 14 patients who underwent palliative esophagectomy were excluded. Five patients who had preoperative AF, defined as a sustained or repetitive arrhythmia documented by electrocardiography (ECG) that required antiarrhythmic therapy [14], were also excluded. Follow-up data were obtained from the database, which included the patients' background, surgical details and tumor characteristics. The tumor invasion (T) and lymph node status (N) were classified by the UICC criteria [15]. Informed consent was obtained from all of the patients in accordance with the guidelines of the Ethics Committee on Human Research of Wakayama Medical University Hospital. Data regarding the patients' age, sex, tumor location, tumor level (TNM stage), cardiac disease, hypertension, preoperative cardiac function test results, preoperative pulmonary function test results, diabetes and treatment with concurrent combined chemotherapy/chemoradiotherapy were analyzed. Patients with conditions such as angina pectoris and previous myocardial infarction were classified as having cardiovascular disease. Poor cardiac function was defined as an ejection fraction <60 %, as measured by echocardiography. Patients with abnormal pulmonary function on spirograms (vital capacity ration [%VC] < 70 % or forced expiratory volume in 1 s [FEV_1]/forced vital capacity [FVC] < 60 %) were classified as having pulmonary disease as a comorbidity [16]. Diabetes mellitus was noted if the patient had a fasting blood glucose concentration >126 mg/dL or was receiving antidiabetic therapy [17]. We administered neoadjuvant chemotherapy with cisplatin and 5-fluorouracil for Stage II–III esophageal cancer. This regimen employed in the Japan Clinical Oncology Group (JCOG) 9907 [18] study is now standard in Japan. In our institute, neoadjuvant chemoradiotherapy (NACRT) has been used to treat localized esophageal carcinoma (cT3–4 tumors). We therefore performed NACRT in the modified JCOG 9906 study with cisplatin and 5-fluorouracil plus concurrent radiotherapy [19].

In our institute, traditional open right transthoracic esophageal resection with two- or three-field lymph node dissection is usually performed for patients with thoracic esophageal cancer. After anterolateral thoracotomy, esophagectomy with regional lymphadenectomy is performed. Our two-field lymph node dissection includes total mediastinal, perigastric and celiac lymphadenectomy. Three-field lymph node dissection adds removal of the lymph nodes in the supraclavicular and cervical paratracheal regions to the two-field approach. In January 2009, we adopted minimally invasive thoracoscopic esophageal resection when the tumor is cT1–2 [20, 21]. In brief, the patient is placed in a prone position, five trocars are placed along the medial edge of the scapula, and thoracoscopic esophagectomy with regional lymphadenectomy is performed. In cases in which a patient has lower third thoracic esophageal cancer with poor cardiac function or has had a previous right thoracotomy, we perform left transthoracic esophageal resections. If the lesion is located in the middle or upper third of the thorax, we use hand-assisted laparoscopic surgery for mobilization of the stomach and abdominal lymph node dissection and gastric tube formation [22].

A gastric conduit through the retrosternal route or through the posterior mediastinum is usually used to construct the anastomosis with the cervical esophagus. In patients with previous gastrectomy or concomitant gastric cancer, the right ileo-colon is used. The esophageal anastomosis is then established in an end-to-side fashion in the cervical wound.

All patients underwent perioperative respiratory rehabilitation, and 125 mg of methylprednisolone was administered intravenously at the beginning of the thoracotomy and again at the end of the surgery [23, 24]. All patients were admitted to the intensive care unit immediately after the operation. All patients began standing and walking on postoperative day (POD)1. Epidural analgesia was used routinely for postoperative pain management for 1 week. All patients having a postoperative cardiac diagnosis by ECG remained under continuous monitoring for at least 72 h after surgery.

In this study, postoperative complications were analyzed according to the Clavien–Dindo classification and the thoracic morbidity and mortality (TM&M) system [25, 26]. In this study, complications higher than Grade II were regarded as clinically significant postoperative complications. Grade II refers to any complication that requires pharmacological treatment or minor intervention only; Grade III means any complication that requires surgical, radiological or endoscopic intervention or multiple therapies (Grade IIIa indicates that intervention does not require general anesthesia; Grade IIIb indicates that intervention requires general anesthesia) and Grade IV indicates any complication requiring intensive care unit management and life support. Surgical mortality (Clavien–Dindo classification Grade V) included in-hospital deaths within 30 days after surgery.

Postoperative AF was defined as an absent P wave before the QRS complex, with irregular ventricular rhythm on the rhythm strips [1, 14]. Leakage at the anastomosis site was determined by the leakage of contrast medium by upper gastrointestinal series after surgery. An intra-abdominal abscess (including bile leakage) was defined as intra-abdominal fluid collection, identified by ultrasonography or computed tomography (CT), with positive cultures. The diagnosis of postoperative pneumonia was made via CT and an elevated white blood cell count and serum C-reactive protein (CRP) level. Respiratory failure was defined as the need for intubation and mechanical ventilation in patients. The diagnosis of postoperative pneumothorax and diaphragmatic hernia was made via chest CT. Postoperative deterioration of liver function was defined by a serum aspartate aminotransferase level more than two times the upper limit of the normal level within 14 days after the operation. The diagnosis of postoperative vocal code paralysis was made via bronchoscopy, which is a routine examination in our group (POD1).

The StatView 5.0 software package (Abacus Concepts, Inc., Berkeley, CA) was used for all statistical analyses. Quantitative results are expressed as medians and ranges. A statistical analysis was performed using Fisher's test. A P value <0.05 was considered to be significant. The univariate and multivariate logistic regression analyses were performed to identify risk factors influencing the development of postoperative AF. Risk factors with a univariate $P < 0.10$ were included in the multivariate analysis. Risk factors with a multivariate $P < 0.05$ were defined as independent risk factors.

Results

Table 1 shows the detailed characteristics of the 207 patients, including 167 males and 40 females, with a median age of 66 years. The tumor was located in the upper third of the thorax in 16 patients (7.7 %), middle third of the thorax in 151 patients (72.9 %) and lower third of the thorax in 40 patients (19.3 %), and the median tumor size was 39 mm. The primary tumors were squamous cell carcinoma (193; 93.2 %), adenocarcinoma (10; 4.8 %) and others (4; 1.9 %). Twenty-five (12.1 %), 34 (16.4 %), 54 (26.1 %), 55 (26.6 %) and 39 (18.8 %) patients had TNM Stage 0, I, II, III and IV, respectively.

Table 2 shows the surgical data. A total of 171 patients (82.6 %) underwent an open right transthoracic esophageal resection. Twenty-eight patients (13.5 %) received a minimally invasive thoracoscopic esophageal resection [21]. Eight patients underwent a left transthoracic esophageal resection. One hundred thirteen patients received a radical three-field lymphadenectomy (total mediastinum, abdomen and cervix), and 88 patients received a two-field lymphadenectomy (total mediastinum and abdomen). After esophagectomy, the stomach was used for reconstruction in 182 patients, the ileo-colon was used in 24 patients and the

Table 1 Clinicopathological features of the patients ($n = 207$)

Age, years (median, range)	66 (43–85)
Sex (male/female)	167/40
Tumor location (Ut/Mt/Lt)	16/151/40
Tumor size, mm (median, range)	39 (3–90)
Pathology (SCC/adenocarcinoma/other)	193/10/4
Depth of invasion[a] (T0/Tis/T1/T2/T3/T4)	2/6/83/35/79/2
TNM stage[a] (0/I/II/III/IV)	25/34/54/55/39
NAC (%)	53 (25.6)
NACRT (%)	14 (6.8)

Ut upper third of the thorax, *Mt* middle third of the thorax, *Lt* lower third of the thorax, *SCC* squamous cell carcinoma, *NAC* neoadjuvant chemotherapy, *NACRT* neoadjuvant chemoradiotherapy

[a] UICC TNM 7th edition

Table 2 Surgical data ($n = 207$)

Approach (right thoracotomy/thoracoscope/left thoracotomy)	171/28/8
Lymph node dissection (3-field/2-field/1-field)[a]	113/88/6
Conduit (stomach/colon/jejunum)	182/24/1
Route of reconstruction (retrosternal/posterior mediastinum)	148/59
Median length of operation, min (range)	525 (310–776)
Median blood loss, mL (range)	437 (45–3,100)
Blood transfusion (%)	54 (26.1)
Curative resection[b] (%)	192 (92.8)

[a] Three-field indicates bilateral cervical regions, the mediastinal space and the abdomen; two-field indicates the mediastinum and the abdomen and one-field indicates the abdomen and the middle/low mediastinum

[b] UICC TNM R0

jejunum was used in one patient. The median duration of the operation was 525 min, and the median blood loss was 437 mL.

The details of the postoperative complications for esophagectomy (more than Grade II morbidity) are listed in Table 3. One or more complications were experienced by 61 patients (29.5 %). AF and pneumonia, classified as Grade II, only required medical therapy (e.g. beta-blockers for AF, or antibiotics for pneumonia). Seventeen patients with lateral vocal cord paralysis were followed through swallowing rehabilitation until recovery. Five patients with bilateral vocal cord paralysis required tracheostomy. Anastomotic leakage was observed in eight patients (3.9 %), six with minor leaks and two with leaks requiring additional drainages. Three patients with respiratory failure required 1–14 days of ventilation and tracheostomy early in the series, and subsequently required 1 month to recover. Diaphragmatic hernias occurred in two patients after esophagectomy with gastric pull-up (one patient with a retrosternal gastric tube, one patient with an intrathoracic gastric tube). These two patients underwent diaphragmatic hernia repair in open surgery. In our series of 207 patients who underwent esophagectomy, no surgical mortality occurred.

Table 3 Postoperative complications ($n = 207$)

Complications	No. (%)	Clavien–Dindo classification[a] (n)		
		Grade II	Grade IIIa	Grade IIIb
Any	61 (29.5)			
Atrial fibrillation	19 (9.2)	19		
Vocal cord paralysis[b]	22 (10.6)	17	5	
Pneumonia	12 (5.8)	12		
Anastomotic leakage	8 (3.9)	6	2	
Pneumothorax	6 (2.9)	6		
Wound infection	5 (2.4)	5		
Respiratory failure	3 (1.4)			3
Chylothorax	2 (1.0)	2		
Cervical lymphatic leakage	2 (1.0)	2		
Enteritis	2 (1.0)	2		
Diaphragmatic hernia	2 (1.0)			2
Deterioration of liver function	1 (0.5)	1		
Bile leakage	1 (0.5)		1	
Intra-abdominal abscess	1 (0.5)		1	
Mortality	0 (0)			

[a] Surgical complications were classified into five categories by the Clavien–Dindo classification

[b] Including transient paralysis

Other complications related to the esophagectomy (more than Grade II morbidity) affected 63.2 % of the patients who had postoperative AF (12/19) compared with 16.0 % of the patients without AF (30/188) ($P < 0.0001$, Table 4). We found that postoperative complications were more common in patients with postoperative AF.

Table 5 shows the details of the patients with postoperative AF. All patients were male, and had a median age of 69 years. In all cases, the postoperative AF occurred within 48 h after surgery. In 12 of the 19 patients (63.2 %), AF was found after patients were up and walking postoperatively. The median duration of postoperative AF was 3.7 days (range 0.5–14 days). All 19 patients received antiarrhythmic drug therapy in the early stage, and 12 patients (63.2 %) responded positively. Successful treatment was defined as a heart rhythm change to a sinus rhythm within 72 h of the start of treatment. In our institute, landiolol hydrochloride, an ultrashort-acting β_1-selective β-blocker has been used as the first-line therapy against postoperative AF since 2002 [12].

The univariate and multivariate analyses were performed to identify risk factors for postoperative AF. Table 6 shows the results of the analysis of the 31 variables that were univariately examined as potential risk factors for the 19 patients with postoperative AF vs the 188 patients without postoperative AF. Nine of the 31 factors differed significantly between these groups ($P < 0.10$). Of the preoperative factors, patients having hypertension ($P = 0.0003$) and having other arrhythmia ($P = 0.0064$) were found to be significant. In terms of the intraoperative factors, ileo-colon use for reconstruction ($P < 0.0001$), reconstruction through the retrosternal route ($P = 0.0870$) and a long operation ($P = 0.0058$) were selected as significant predictors. Among the various postoperative factors, dopamine use ($P = 0.0484$), the onset of other complications ($P < 0.0001$), tachycardia ($P < 0.0001$) and fever ($P = 0.0041$) were selected. The multivariate logistic regression analysis indicated that postoperative AF was significantly associated with the use an ileo-colon for reconstruction after esophagectomy ($P = 0.0023$) and the presence of tachycardia with a heart rate of >100 bpm on POD1 ($P = 0.0004$), with odds ratios of 13.6 (95 % confidence interval [CI], 2.5–72.4) and 18.4 (95 % CI, 3.7–92.0), respectively.

Table 4 Correlation between the onset of postoperative atrial fibrillation and other complications in patients after esophagectomy ($n = 207$)

Other complications	Atrial fibrillation		
	Yes ($n = 19$)	No ($n = 188$)	P value
Yes ($n = 42$)	12	30	<0.0001
No ($n = 165$)	7	158	

We found that postoperative AF was significantly associated with the use of a colon conduit for reconstruction and with tachycardia on POD1 (Table 6). Therefore, we examined the incidence of postoperative AF in patients with these factors. As shown in Table 7, the incidence of postoperative AF in patients with both colon conduit use for reconstructions after esophagectomy and those who had tachycardia with a rate >100 bpm on POD1 was 100 % (4/4), while the incidence of postoperative AF in patients without these two factors was only 2.4 % (4/168).

Discussion

Major pulmonary complications and surgical sepsis are common morbidities in patients with AF after esophagectomy [6]. The association of postoperative AF with mortality has also been documented after lung surgery [27] and after major noncardiac operations [14]. In the present study, postoperative complications were also frequently observed in patients with AF.

We speculate that AF after esophagectomy is caused by an inflammatory response following surgical trauma to the sympathovagal nerve fibers supplying the heart [2, 28, 29]. It has been reported that advanced age and a history of cardiac disease are predisposing factors for postoperative AF [6]. Ma et al. [2] reported that AF was associated with postoperative hypoxia, a history of COPD, thoracic–gastric dilatation, an age older than 65 years, male sex and a history of cardiac disease. Stippelet al [30] reported that an elevated body temperature was the most important predisposing factor for AF. Interestingly, Hou et al. [1] described that an elevated level of perioperative N terminal (NT)-pro B-type natriuretic peptide (BNP) is an independent predictor of AF. Thus, previous studies have shown different results.

We found that the use of colonic interposition for esophageal replacement after esophagectomy was a predictor of postoperative AF. This association has not been

Table 5 Summary of patients with postoperative atrial fibrillation

Patient no.	Age	Sex	Onset of an AF	Situation at the onset	Duration of AF (days)	Drugs	Other complications
1	72	M	POD2	Resting	0.5	Digoxin	None
2	68	M	POD2	Resting	0.5	Digoxin	Pneumonia
3	71	M	POD2	Resting	2	Digoxin	None
4	68	M	POD2	Resting	4	Digoxin	Pneumothorax
5	85	M	POD2	After walking	6	Digoxin Landiolol Verapamil	Enterocolitis
6	77	M	POD2	After walking	10	Landiolol Pilsicainide Verapamil	Respiratory failure
7	71	M	POD1	After walking	4	Digoxin Landiolol Verapamil	Pneumonia
8	68	M	POD1	Resting	1	Landiolol	Vocal cord paralysis
9	52	M	POD2	Resting	0.5	Pilsicainide	Vocal cord paralysis
10	64	M	POD2	After walking	1	Landiolol	None
11	72	M	POD2	After walking	3	Landiolol Disopyramide	None
12	76	M	POD2	After walking	2	Landiolol	Anastomotic leakage
13	75	M	POD2	After walking	14	Landiolol	Anastomotic leakage Intra-abdominal abscess
14	67	M	POD2	Resting	3	Landiolol	None
15	65	M	POD2	After walking	6	Pilsicainide	Vocal cord paralysis
16	71	M	POD1	After walking	2	Landiolol	None
17	65	M	POD1	After walking	2	Landiolol	Pneumothorax
18	59	M	POD1	After walking	7	Landiolol	None
19	62	M	POD2	After walking	2	Pilsicainide	Vocal cord paralysis

AF atrial fibrillation, *POD* postoperative day, *M* male

Table 6 Results of the univariate and multivariate analyses of risk factors influencing the postoperative atrial fibrillation

Risk factors	Categories	Univariate analysis	Multivariate analysis	
		P value	*P* value	Odds ratio (95 % CI)
Preoperative factors				
Sex	Male	0.9668		
	Female			
Age (years)	≥70	0.2932		
	<70			
BMI (kg/m^2)	≥25	0.9666		
	<25			
Cancer location	Ut	0.9793		
	Mt/Lt			
NAC	Yes	0.5326		
	No			
NACRT	Yes	0.4979		
	No			
History of cardiovascular disease	Yes	0.1864		
	No			
History of hypertension	Yes	0.0003	0.0648	3.660 (0.923–14.510)
	No			
History of pulmonary disease	Yes	0.2573		
	No			
History of diabetes mellitus	Yes	0.7009		
	No			
Ejection fraction (%)	<60	0.5659		
	≥60			
R(L)BBB, PVC or AV block	Yes	0.0064	0.1410	3.289 (0.674–16.057)
	No			
Intraoperative factors				
Approach	Thoracotomy	0.1068		
	Thoracoscope			
Lymphadenectomy	3-Field	0.1099		
	2-Field			
Conduit	Colon	<0.0001	0.0023	13.580 (2.546–72.426)
	Gastric tube/jejunum			
Route of reconstruction	Retrosternal	0.0870	0.3277	2.727 (0.366–20.338)
	Posterior mediastinum			
Thoracic duct resection	Yes	0.9786		
	No			
Duration of operation (min)	≥600	0.0058	0.6799	1.384 (0.295–6.484)
	<600			
Blood loss (mL)	≥500	0.1917		
	<500			
Blood transfusion	Yes	0.2674		
	No			
Curative resection[a]	Yes	0.7278		
	No			
Postoperative factors				
Dopamine use	Yes	0.0484	0.6966	0.746 (0.171–3.249)
	No			