

Prognostic significance of circulating tumor cells in patients with advanced esophageal cancer

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Abstract

Introduction The purpose of this study was to establish the clinicopathological significance of circulating tumor cells (CTCs) in patients with advanced or recurrent esophageal cancer who received either chemotherapy or chemoradiotherapy.

Methods CTCs from 38 patients with advanced or recurrent esophageal cancer were quantified using the CellSearch system before and approximately 3–5 weeks after the initiation of a new line of chemotherapy or chemoradiotherapy. The correlation between CTC counts and clinicopathological variables was examined.

Results Of the 38 patients, 15 (39 %) had recurrent esophageal cancer, and 23 patients (61 %) had primary advanced esophageal cancer. The mean age was 63 years (range 43–87 years). One patient (2.6 %) had a complete response to treatment, 16 (42.1 %) had a partial response, 12 (31.6 %) showed stable disease, and nine (23.7 %) showed signs of progressive disease. The overall survival of patients with ≥ 2 CTCs was significantly shorter than that of patients with < 2 CTCs both at baseline and at first follow-up ($p = 0.047$ and $p = 0.011$, respectively). Significant correlation was found between the change in CTC counts and the response to treatment ($p = 0.036$). The overall survival in patients with ≥ 2 CTCs both at baseline and at first follow-up was significantly shorter than patients with < 2 CTCs both at baseline and at first follow-up ($p = 0.002$). Patients with a reduction rate in the CTC value < 80 %

experienced significantly shorter survival than patients with a reduction rate of ≥ 80 % ($p = 0.035$). The CTC counts at first follow-up served as an independent prognostic factor ($p = 0.011$).

Conclusions Our results suggest that measuring the number of CTCs in patients with advanced or recurrent esophageal cancer can be useful for predicting the survival of patients and for monitoring the response to cancer treatments.

Keywords Circulating tumor cells · Esophageal cancer · CellSearch system

Introduction

The existence of circulating tumor cells (CTCs) has long been recognized. Indeed, numerous reports have described the isolation and characterization of CTCs from patients with various types of carcinomas [1–8]. Despite this, detection of CTCs in peripheral blood requires highly accurate, specific, and reproducible methods. To date, immunocytochemistry [9], reverse-transcription polymerase chain reaction (RT-PCR) [10], and flow cytometry [11] have all been used for the isolation of CTCs from peripheral blood.

The CellSearch System (Veridex LLC, Warren, NJ) was designed to detect tumor cells in whole blood [12]. This system has made it possible to count CTCs accurately and reproducibly even in different laboratories. Quantification of CTCs in metastatic breast cancer using the CellSearch system has been reported to represent an earlier and more reproducible indicator of disease status than current imaging methods [13]. Furthermore, Allard et al. [12] reported that CTCs are detectable in patients with various types of metastatic carcinomas, including gastrointestinal cancers.

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Cohen et al. [14] reported that the presence of CTCs before the initiation of therapy and at follow-up is a strong and independent prognostic indicator of both poorer progress-free and overall survival. Indeed, Seeberg et al. [15] recently reported that CTC counts in patients with colorectal cancer also predict non-resectability and impaired survival.

Esophageal cancer is the eighth most frequently diagnosed cancer and the sixth leading cause of cancer deaths worldwide, with an estimated 482,000 new cases and 407,000 deaths reported in 2008. Additionally, this cancer is well known for its early metastasis to lymph nodes and for recurrence, which results in poor prognosis [16, 17]. Despite improvements in detection, surgical resection, and (neo) adjuvant therapy, overall survival associated with esophageal cancer remains worse in comparison with other solid tumors [18, 19]. In Japan, esophagectomy with radical lymphadenectomy has been the main potentially curative treatment for patients with esophageal cancer [20]. Chemotherapy or chemoradiotherapy including neoadjuvant therapy has also proved to be an effective treatment, particularly in patients with advanced or recurrent esophageal cancer.

We previously reported that the quantification of CTCs in patients with gastrointestinal cancer including esophageal cancer can be a useful tool for tumor staging and for predicting both patient survival and the presence of peritoneal or pleural dissemination. In addition, we demonstrated that CTC counts are significantly higher in patients with gastrointestinal cancer than in healthy donors [21].

Our hypothesis is that the number of CTC in patients with advanced or recurrent esophageal cancer is associated with prognosis and a response to treatment. In this study, we quantified CTCs before the initiation of a new line of chemotherapy or chemoradiotherapy (at baseline) and approximately 3–5 weeks after the initiation of the treatment (at first follow-up); we tested whether CTC counts correlate with prognosis and a response to treatment.

Patients and methods

Study design

Between March 2010 and March 2012 at Keio University Hospital, we enrolled patients with either cT2–4b advanced esophageal cancer histopathologically diagnosed using endoscopic biopsy [Union for International Cancer Control TNM (tumor-nodes-metastasis) classification, 7th edition] or with recurrent esophageal cancer who were about to start with a new line of chemotherapy or chemoradiotherapy. All patients had an Eastern Cooperative Oncology Group performance status score of 0–1. A complete

review of the medical histories and physical examinations were performed for all patients prior to treatment. The required staging assays included an endoscopy and a computed tomography (CT) scan of the cervical, chest, abdominal, and pelvic areas. The number of CTCs was measured at baseline and after a period of approximately 3–5 weeks following the initiation of a new line of therapy (at first follow-up). All cases were followed prospectively and enrolled using a protocol approved by the Institutional Review Board of Keio University Hospital. All patients read and signed informed consent forms.

Imaging

CT scans of the cervical, chest, abdominal, and pelvic areas were planned to be performed at baseline and approximately 2 months after the initiation of a new line of treatment. Image interpretation was performed using Response Evaluation Criteria in Solid Tumors (RECIST) to classify each disease assessment as either a complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) [22]. In cases with no measurable lesions, the primary lesion was measured using upper gastrointestinal endoscopy and upper gastrointestinal series.

CTC quantification

We drew 7.5 ml of blood into CellSave Preservative Tubes (Veridex LLC, Raritan, NJ). The samples were stored at room temperature and processed within 72 h of collection. All assessments were performed in our laboratory in the Department of General and Gastrointestinal Surgery, Keio University School of Medicine without knowing the clinical status of the patients. The CTC quantification procedure involved a semi-automated system for the processing of samples using the CellSearch Epithelial Cell Kit. The procedure involves the enrichment of a blood sample with cells expressing EpCAM using antibody-coated magnetic beads, followed by staining cell nuclei with the fluorescent nucleic acid dye 4,2-diamidino-2-phenylidole dihydrochloride (DAPI). Fluorescent-labeled monoclonal antibodies specific to leukocytes (allophycocyan-anti-CD45) and to epithelial cells (8,19,19-phycoerythrin-anti-cytokeratin) were used to distinguish epithelial cells from leukocytes. Identification and quantification of CTCs were performed by means of the Cell Spotter and Cell Tracks Analyzer, a semi-automated fluorescence-based microscopy system that allows for computer-generated reconstruction of cellular images. CTCs were defined as nucleated cells lacking CD45 but expressing cytokeratin. The criteria used in the CellSearch system to define a tumor cell were described previously [1]. Results were expressed as the number of cells per 7.5 ml of whole blood. Technical details on the

CellSearch and Cell Spotter systems, including accuracy, precision, linearity, and reproducibility, have previously been described by Allard et al. [12]. Allard et al. reported that eight of 145 healthy donors had 1 CTC per 7.5 mL of blood, and no healthy donors had ≥ 2 CTCs per 7.5 mL of blood. In fact, we also reported in our previous study that no healthy donors had ≥ 2 CTCs [21]. Based on these reports, we considered ≥ 2 CTCs per 7.5 mL of blood to represent a positive result.

Definition of changes in CTC counts

In this study, we classified all changes in CTC counts into three groups: increasing, stable, and decreasing. A positive (above threshold) CTC result at first follow-up after a negative CTC result at baseline was regarded as increasing. A negative CTC result at first follow-up after a positive CTC result at baseline was regarded as decreasing. A negative CTC result both at baseline and at first follow-up was regarded as stable. In case of a positive CTC result both at base line and at first follow-up, an increase of ≥ 2 CTCs was regarded as increasing, a decrease of ≥ 2 CTCs was regarded as decreasing, and a change of < 2 CTCs was classified as the stable group.

Statistics

To evaluate the correlation of the changes in CTC counts with a response to treatment and the distribution of the patients with ≥ 2 and < 2 CTCs at baseline, data were analyzed using Fisher's exact test. Survival analysis was performed using the product limit Kaplan–Meier and log-rank tests. Multivariate analysis was conducted using the Cox proportional hazards model, with testing for independent prognostic variables. All calculations were carried out using SPSS software, version 21 for Windows (SPSS Japan Inc., Tokyo, Japan). A p value of < 0.05 was assumed to denote statistical significance.

Results

Patient's characteristics

We analyzed data from 38 patients with advanced or recurrent esophageal cancer who were enrolled in this study. Patient characteristics are detailed in Table 1. The mean age was 63 years, ranging from 43 to 87 years. There were 30 males and 8 females, and the median follow-up was 19 months.

Among the 38 patients, positive CTC results (≥ 2 CTCs) were observed in 19 patients (50 %) at baseline and in 15 (39 %) at first follow-up; CTC counts ≥ 3 were observed in 10 patients (26 %) at baseline and in 10 (26 %) at first

follow-up. Of the 38 patients, three received a diagnosis of adenocarcinoma after an endoscopic biopsy, 34 received a diagnosis of squamous cell carcinoma, and one received a diagnosis of basaloid squamous cell carcinoma. Twenty-three patients were newly diagnosed with advanced esophageal cancer, and 15 were diagnosed with recurrent esophageal cancer. Of the 23 patients with newly diagnosed advanced esophageal cancer, five had a cT2 tumor, 13 had a cT3 tumor, three had a cT4a tumor, and two had a cT4b tumor. Of those 23 patients, three patients were diagnosed with stage I cancer, two with stage II, 15 with stage III, and three with stage IV cancer. Of the three patients with stage IV cancer, one patient had metastatic lesions in an adrenal gland and a lung, another had bone metastases, and the third patient had no regional lymph node metastasis. Among the 15 patients with recurrent esophageal cancer, measurable lesions involved anastomotic recurrence and metastasis to the lymph nodes, kidney, liver, or bone.

Therapeutic effects

In this study, 17 patients received chemotherapy and 21 received chemoradiotherapy. The chemotherapy regimens included 5-fluorouracil/cisplatin, docetaxel/5-fluorouracil/cisplatin, 5-fluorouracil/nedaplatin, docetaxel/nedaplatin, or S-1/cisplatin. The chemoradiotherapy regimen included either 5-fluorouracil/cisplatin or S-1/cisplatin. One patient (3 %) showed CR, 16 (42 %) showed PR, 12 (32 %) showed SD, and 9 patients (23 %) showed PD.

The correlation between the changes in CTC counts and therapeutic effects

We categorized all the patients into two groups on the basis of therapeutic outcome. One group (CR/PR group) comprised the patients whose therapeutic outcome was either CR or PR, and the other group was either SD or PD. In addition, we divided all the patients into two groups on the basis of the abovementioned definition of changes in CTC counts. One group comprised patients whose CTC counts were either increasing or stable, and the other group comprised the patients whose CTC counts were decreasing. The correlation between the change in CTC counts and therapeutic effect is shown in a 2×2 table (Table 2). In this table, a significant correlation was found between the change in CTC counts and the response to treatment (Fisher's exact test, $p = 0.036$).

The background data of the patients with ≥ 2 and < 2 CTCs at baseline

We compared the background data of patients with ≥ 2 and < 2 CTCs at baseline (Table 1). We evaluated the clinical

Table 1 Clinicopathological features of patients in this study

	Overall		CTCs at baseline				<i>p</i>
	<i>n</i> = 38	%	<2	%	≥2	%	
Male	30	79	16	53	14	47	0.693
Female	8	21	3	38	5	63	
Median age (years) (range)	63 (43–87)		63 (55–73)		64 (43–87)		0.977
Performance status							
0	38	100	19	50	19	50	1.000
1	0	0	0		0		
Pathological diagnosis							
Adenocarcinoma	3	8	2	67	1	33	0.513
Squamous cell carcinoma	34	89	17	50	17	50	
Other	1	3	0	0	1	100	
Therapeutic effect							
CR	1	2	0	0	1	100	0.343
PR	16	42	8	50	8	50	
SD	12	32	8	67	4	33	
PD	9	24	3	33	6	67	
Regimen of therapy							
Chemotherapy							
FP	11	29	7	64	4	36	0.324
DCF	6	16	3	50	3	50	
Neda/Doce	3	8	0	0	3	100	
Neda/5-FU	1	3	0	0	1	100	
Chemoradiotherapy							
FP	16	41	8	50	8	50	
S1/CDDP	1	3	1	100	0	0	
Advanced esophageal cancer							
T factor (UICC 7th edition)							
cT2	5	22	3	60	2	40	0.838
cT3	13	56	8	62	5	38	
cT4a	3	13	1	33	2	67	
cT4b	2	9	1	50	1	50	
Stage							
IB	3	13	2	67	1	33	0.739
IIA	1	4	1	100	0	0	
IIB	1	4	1	100	0	0	
IIIA	5	26	2	40	3	60	
IIIB	4	17	3	75	1	25	
IIIC	6	22	3	50	3	50	
IV	3	13	1	33	2	67	
Recurrent esophageal cancer							
Distant metastasis (–)	11	73	4	36	7	64	1.000
Distant metastasis (+)	4	27	2	50	2	50	
Recurrent site							
Lymph node	10		4	40	6	60	
Liver	1		0	0	1	100	
Bone	1		1	100	0	0	
Peritoneal	2		0	0	2	100	
Anastomosis	2		1	50	1	50	

CR complete response, PR partial response, SD stable disease, PD progressive disease, FP 5-FU + cisplatin, DCF docetaxel + cisplatin + 5FU, Neda/Doce nedaplatin + docetaxel, Neda/5-FU nedaplatin + 5-FU, S1/CDDP TS-1 + cisplatin

Table 2 The correlation between changes in circulating tumor cell (CTC) counts and therapeutic effects

	The change in CTC counts	
	Decreasing	Increasing and stable
CR/PR	8	9
SD/PD	3	18
		<i>p</i> = 0.037

The changes in CTC counts are defined in the methods

CR/PR the group of the patients with complete response or partial response, SD/PD the group of patients with either stable or progressive disease

T factor and clinical stage status in patients with advanced esophageal cancer and the distant metastatic status in patients with recurrent esophageal cancer. Between the patients with ≥ 2 and < 2 CTCs at baseline, no significant differences were observed in either the clinical T factor or clinical stage status of patients with advanced esophageal cancer or in the distant metastatic status of the patients with recurrent esophageal cancer.

Overall survival at baseline and at first follow-up

The overall survival of patients with ≥ 2 CTCs at baseline and at first follow-up was significantly shorter than that of patients with < 2 CTCs ($p = 0.047$ and $p = 0.011$, respectively; Fig. 1). Moreover, the overall survival of patients

with ≥ 3 CTCs at first follow-up was significantly shorter than that of patients with < 3 CTCs ($p = 0.011$, data not shown), although no significant differences were observed in the overall survival between patients with ≥ 3 CTCs and < 3 CTCs at baseline ($p = 0.249$, data not shown). Patients with ≥ 3 CTCs at first follow-up had a significantly poorer prognosis compared with patients with < 3 CTCs in the recurrent esophageal cancer group ($p = 0.002$, data not shown).

Overall survival in the chemotherapy and chemoradiotherapy groups

We divided the 38 patients into two groups. One group was treated with chemotherapy (the chemotherapy group) and the other with chemoradiotherapy (the chemoradiotherapy group). Overall survival curves in patients with ≥ 2 CTCs in both groups tended to be lower than those in patients with < 2 CTCs at both baseline and first follow-up. In particular, patients with ≥ 2 CTCs at first follow-up in the chemoradiotherapy group had a significantly shorter survival than those with < 2 CTCs ($p = 0.007$, data not shown).

In addition, we also analyzed 11 of the 38 patients who had either inoperable or recurrent esophageal cancer and were treated solely with chemotherapy. Of the 11 patients, those with ≥ 2 CTCs at first follow-up ($n = 3$) demonstrated a trend toward poorer overall survival compared with patients with < 2 CTCs ($n = 8$) (1-year overall survival in patients with ≥ 2 CTCs, 0 %; patients with < 2 CTCs, 60 %), despite no significant difference being observed.

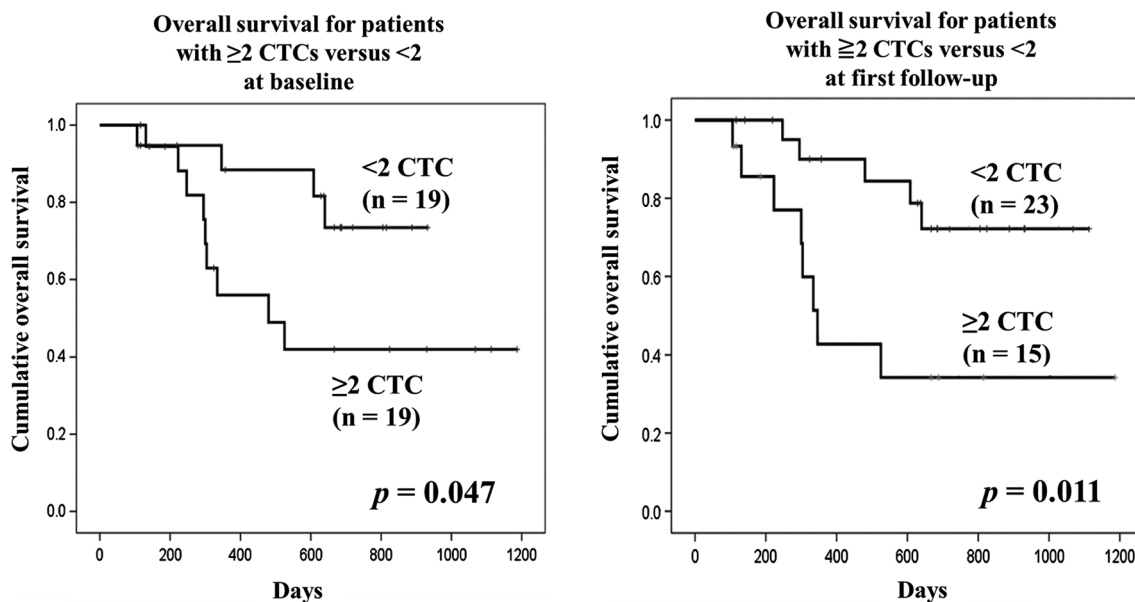


Fig. 1 Overall survival of patients with ≥ 2 circulating tumor cells (CTCs) vs < 2 CTCs: **a** at baseline, and **b** at first follow-up

Overall survival and imaging results

To test whether the therapeutic outcomes evaluated using Recist were associated with patient survival, we constructed cumulative survival curves for the CR/PR and SD/PD groups. No significant differences were observed in the overall survival between these two groups ($p = 0.171$, data not shown).

Prediction of overall survival by means of the change in CTC counts from baseline to first follow-up

We categorized all the patients into four groups on the basis of their CTC counts at baseline and at first follow-up. Group 1 comprised patients with <2 CTCs, both at baseline and at first follow-up, group 2 included patients with ≥ 2 CTCs at baseline and <2 CTCs at first follow-up, group 3 included patients with <2 CTCs at baseline and ≥ 2 CTCs at first follow-up, and group 4 consisted of patients with ≥ 2 CTCs both at baseline and at first follow-up. The overall survival curves of groups 1, 2, 3, and 4 are shown in Fig. 2. The overall survival in group 4 was significantly shorter than that in group 1 ($p = 0.002$; Fig. 2). Moreover, the overall survival curves of groups 2 and 3 were below the curve of group 1 and above that of group 4.

The correlation between the reduction rate in CTC value before and after treatment and prognosis

Of the 38 patients, CTC counts decreased in 14 patients, and of these patients, the average reduction rate was 83 %, with a range of 50–100 %. In addition, we divided the 38 patients into two groups, with one group comprising patients with a reduction rate in the CTC value of ≥ 80 %, and the other group comprised patients with a reduction rate of <80 %. Patients with a reduction rate in the CTC value <80 % experienced significantly shorter survival than patients with a reduction rate of ≥ 80 % ($p = 0.035$; Fig. 3).

Multivariate analysis of predictors of overall survival

Univariate and multivariate analysis using Cox proportional hazards regression was performed to evaluate the association between factors of interest and overall survival. Results are summarized in Table 3. The CTC counts at first follow-up served as an independent prognostic factor.

Discussion

In this study, we tested whether CTC counts in 38 patients with advanced or recurrent esophageal cancer are associated with prognosis and a therapeutic response. Our data

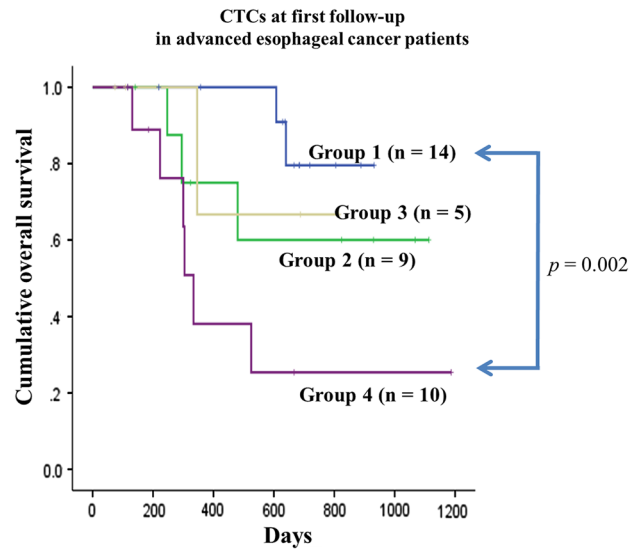


Fig. 2 Circulating tumor cell counts of <2 both at baseline and at first follow-up were significantly associated with longer overall survival. CTC counts of ≥ 2 at both time points were significantly associated with shorter overall survival. The figure shows overall survival of these four groups: patients with <2 CTCs both at baseline and at first follow-up; patients with ≥ 2 CTCs at baseline and <2 CTCs at first follow-up; patients with <2 CTCs at baseline and ≥ 2 CTCs at first follow-up; and patients with ≥ 2 CTCs both at baseline and at first follow-up

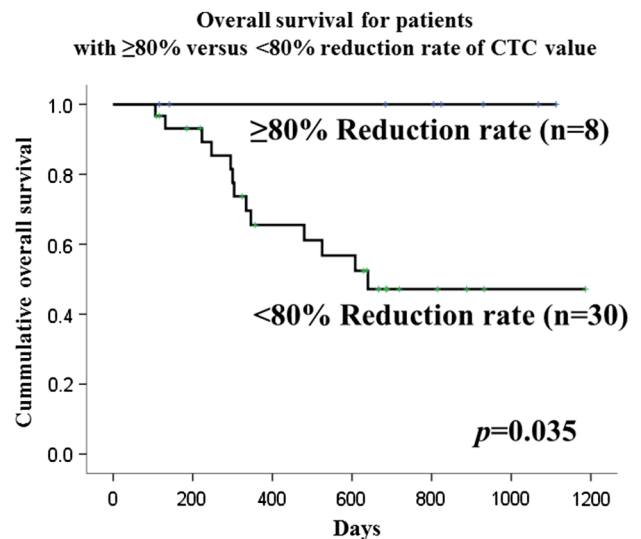


Fig. 3 Overall survival of patients with a reduction rate CTC value ≥ 80 vs <80 %, before and after new line therapy

show that patients with positive (above threshold) CTC results have a significantly worse prognosis than patients with negative CTC results. Indeed, our report seems to be the first to show that CTC counts in patients with advanced or recurrent esophageal cancer are significantly associated

Table 3 Multivariate cox regression analysis for predicting overall survival

Variable	Univariate <i>P</i> value	Multivariate <i>P</i> value	Hazard ratio	95 % CI
Gender, male vs female	0.270			
Age at diagnosis, ≥ 63 vs < 63	0.755			
Stage, IB-III A vs IIIB-IV	0.018	0.034	9.301	1.179–73.406
Therapeutic response, CR/PR vs SD/PD	0.171			
CTC at first follow-up, ≥ 2 vs < 2	0.011	0.011	4.440	1.404–14.041

CI confidence interval

with clinical prognosis and, moreover, a significant correlation exists between the change in CTC number and therapeutic outcome.

In this study, the overall survival of patients with ≥ 2 CTCs, both at baseline and at first follow-up, is significantly shorter compared to the patients with < 2 CTCs. Furthermore, overall survival of patients with ≥ 3 CTCs at first follow-up was significantly shorter than that of patients with < 3 CTCs. According to these results, CTC counts at first follow-up appear to serve as a better prognostic factor for patients with advanced or recurrent esophageal cancer (compared to CTC counts at baseline). Hayes et al. [23] reported that the overall survival of patients with metastatic breast cancer with ≥ 5 CTCs at any point is significantly shorter compared to < 5 CTCs. We also evaluated the overall survival when comparing patients with ≥ 4 and < 4 CTCs in this study, although no significant differences were observed. Of the 38 patients, seven had ≥ 4 CTCs at baseline whilst only four had ≥ 4 CTCs at first follow-up. The sample size and the number of patients with ≥ 4 CTCs were too small for this comparison between patients with ≥ 4 and < 4 CTCs to be valid.

In addition, we evaluated the overall survival in the chemotherapy and the chemoradiotherapy groups. Overall survival curves in patients with ≥ 2 CTCs in both groups tended to be lower than those with < 2 CTCs, and, in particular, overall survival curves in patients with < 2 CTCs in the chemoradiotherapy group had a significantly shorter survival than those with < 2 CTCs. In the case of the analysis of the 11 patients with either inoperable or recurrent esophageal cancer in the chemotherapy group, patients with ≥ 2 CTCs at first follow-up demonstrated a trend toward a poorer overall survival (1-year survival in patients with ≥ 2 CTCs, 0 %; in patients with < 2 CTCs, 60 %), despite a significant difference not being detected. One possibility to explain this is that the sample size may be too small to detect significant differences when the patients were divided into two or more groups.

In the present study, approximately 3–5 weeks passed between evaluating the CTC counts at baseline and evaluation of CTC counts at first follow-up. Compared to breast cancer, advanced or recurrent esophageal cancer tends to progress more rapidly; therefore, immediate measurement

of CTC counts is more important for the prediction of patient survival. With regard to the timing of first follow-up, we selected a period of approximately 3–5 weeks after the initiation of therapy. We usually evaluate the therapeutic outcome in the outpatient clinic before the second cycle of therapy. In a clinical setting, it is very difficult to draw blood from every patient at the exact same point after initiation of therapy.

Furthermore, in several reports, 3–5 weeks was selected as the time point for blood to be drawn at the first follow-up [14, 24]. Indeed, Cohen et al. [14] also measured the number of CTCs in patients with metastatic colorectal cancer at baseline and 3–5 weeks after the initiation of therapy. They utilized the receiver operating characteristic curve to select the optimal time point to draw blood after therapy initiation from a set of different time points including 1–2, 3–5, 6–12, and 13–20 weeks. From this analysis, the 3–5 weeks blood draw time point provided the largest area under the receiver operating characteristic curve. In addition, Cristofanilli et al. [24] also collected the first follow-up visit blood sample from patients with metastatic breast cancer approximately 3–4 weeks after the initiation of new therapy.

We evaluated the response to a new line of chemotherapy or chemoradiotherapy using CT according to RECIST guidelines approximately 6 weeks after the initiation of treatment. In this study, a significant correlation was found between changes in CTC counts and the therapeutic outcome. Nonetheless, no significant differences in the overall survival between the CR/PR group and SD/PD group were observed. In contrast, a significant correlation was found between CTC counts and the patient prognosis.

Measurement of CTC counts in patients with advanced or recurrent esophageal cancer may be an earlier and more sensitive prognostic biomarker compared to traditional imaging methods and a tumor marker such as SCC. Budd et al. [13] reported that the median value of overall survival for patients with metastatic breast cancer with no radiologic progression and ≥ 5 CTCs was significantly shorter than that of patients with no radiologic progression and < 5 CTCs. Thus, the authors concluded that quantification of CTCs is an earlier, more reproducible indicator of prognosis than current imaging methods. The strong correlation

between CTC counts and clinical prognosis suggests that CTC counts may therefore represent an earlier and more sensitive prognostic biomarker compared with RECIST for evaluation of therapeutic outcomes.

The limitation of this study is the small sample size. Indeed, larger, multicenter prospective studies should be conducted to confirm the utility of CTC counts as a prognostic biomarker in patients with advanced or recurrent esophageal cancer. This type of cancer progresses rapidly even during treatment and the washout period of chemo (radio) therapy. Quantification of CTCs may help to quickly identify patients with a poor prognosis, and this timely information may prompt physicians to stop current therapy and start a possibly more effective, multimodal treatment of these patients.

Ethical Statement This study was approved by the Ethics Committee of Keio University School of Medicine and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

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