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Clinical significance of tumor cells in the peripheral blood of patients with esophageal squamous cell carcinoma

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Abstract

Circulating tumor cells (CTCs) are suspected of predicting the prognosis of malignant tumor, but there are few relevant reports specific to esophageal squamous cell carcinoma (ESCC). This study investigated the clinical significance of CTCs in patients with ESCC.

Sixty patients with ESCC were enrolled, from whom CTCs had been tested by our team previously. Peripheral blood samples were obtained from these patients before treatment; and CTCs were assayed by isolation by size of epithelial tumor cells (ISET). Associations between the presence of CTCs and patients' clinicopathological parameters and clinical outcomes were analyzed.

CTCs were detected in 20 patients (33.3%), who experienced significantly shorter progression-free survival (PFS) than did the CTC-negative patients. Overall, PFS was negatively associated with the number of CTCs. Multivariate analyses showed that a CTC count >2 was a strong independent prognostic indicator of tumor recurrence (hazard ratio [HR] 5.63; 95% confidence interval [CI] 1.77–17.89; $P = .003$). In the subgroup of 50 patients who underwent R0 resection and postoperative adjuvant radiotherapy or chemotherapy, CTC was a strong, independent, and prognostic indicator of tumor recurrence (HR 10.70; 95% CI, 1.40–81.91; $P = .022$). The number of CTCs correlated with the T stage ($r = 0.26$, $P = .043$) but not with the N or M stage. For subgroups in stages II or I-IIIB or T3 or T3+T4, the PFS of patients with CTCs > 1 or > 2 was significantly shorter than that of the patients with CTCs ≤ 1 or CTCs ≤ 2. In the stage III or T3+T4 groups, the PFS of patients with CTCs > 0 was significantly shorter than that of patients with CTC = 0.

This is the first study to report that the CTC detected by ISET is an independent and prognostic indicator of patients' outcome in ESCC. Consideration of CTCs may improve the accuracy of preoperative staging in ESCC.

Abbreviations: CEA = carcinoembryonic antigen, CI = confidence interval, CTCs = circulating tumor cells, ESCC = esophageal squamous cell carcinoma, HR = hazard ratio, ISET = isolation by size of epithelial tumor cells, PFS = progression free survival, WHO = World Health Organization.

Keywords: circulating tumor cells, clinical significance, esophageal squamous cell cancer, ISET, prognostic value

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1. Introduction

Esophageal carcinoma is a common and deadly cancer in China and ranked as the fourth cause of cancer-related deaths. Since the early symptoms of esophageal squamous cell carcinoma (ESCC) are often hard to recognize, patients are usually at an advanced stage at the initial diagnosis. Late treatment leads to a poor prognosis, with 5-year survival rates of only 15% to 25%.[1–3] A poor prognosis is usually due to recurrence and metastasis.

Clinically, a considerable number of early-stage ESCC patients do not have obvious metastasis, but die of early tumor recurrence and distant metastasis after radical surgery.[4] This suggests that the spread of cancer cells cannot be detected by conventional clinical or histopathological methods.

Circulating tumor cells (CTCs) are tumor cells that are released into the peripheral blood from the primary tumor or metastatic lesions, either spontaneously or due to surgery.[5] CTCs from various tumors have the potential to act as precursors of metastases, including esophageal cancer.[6,7] Methods to detect CTCs in esophageal cancer are mainly the following: reverse transcription (RT)-PCR, the Celltracks AutoPrep system, and isolation by size of epithelial tumor cells (ISET). RT-PCR disrupts the cells and is not applicable to a small number of CTCs, and the Celltracks AutoPrep system is limited by its inability to detect nonepithelial CTCs, which leads to a low detection rate. Although ISET technology loses a few small CTCs (<8 μm diameter), the technique is simple, inexpensive, and capable of separating viable CTCs. Thus, ISET is an ideal technique to detect CTCs of esophageal cancer.

The detection of CTCs is important for guiding the treatment strategy, and has been confirmed for prognostic evaluation of breast, prostate, gastric cancer, and other common malignant tumors.[8–10] However, there are few studies regarding the clinical significance of CTCs in ESCC, and most relevant studies used the Celltracks AutoPrep system for evaluations.

The present study investigated the clinical significance of CTCs in patients with ESCC. ISET was to detect the CTCs of ESCC patients before treatment.[11]

2. Patients and methods

2.1. Patients

The Shandong Provincial Cancer Research Institute Ethics Committee approved this study. All the enrolled patients provided written consent.

The inclusion criteria of the present study were the following: age >18 years; histological diagnosis of ESCC; World Health Organization (WHO) performance status between 0 and 2; and, treated only once or were without treatment for at least 6 months. Patients with any of the following were excluded: a history of unrelated carcinoma in the preceding 5 years; a history of dermatologic disease; or cervical esophageal cancer.

From May to December 2014, a cohort of 60 consecutive patients with primary ESCC was studied, which constituted the same cohort of patients who participated in our previous study,[11] except for 1 patient who withdrew from this follow-up study by his own initiative. Of the 60 patients, 50 patients had undergone R0 resection and postoperative adjuvant radiotherapy or chemotherapy, and were analyzed as a subgroup (surgery + chemoradiotherapy). Excluded were the remaining 10 patients with distant organ metastatic lesions and treated only with radiotherapy and chemotherapy and without surgical resection.

2.2. CTC analysis

CTC analysis was completed in our previous study using the CTC BIOPSY system to detect peripheral blood CTCs in a cohort of 61 patients.[11] Because in the present study 1 patient did not meet the inclusion criteria and was excluded, only the data of the 60 remaining patients were used for further analysis. The ISET assay was performed as described by Vona et al.[12] The samples were processed on an automatic testing platform in accordance with the manufacturer's instructions. Five milliliters of whole blood were diluted up to 8 mL with buffer containing 0.2% formaldehyde, and filtered through a membrane with a pore size of 8 mm. The harvested CTCs and circulating tumor microemboli were stained with Romanowsky stain, air dried, mounted, and blindly reviewed independently by 3 senior cytopathologists.

The isolated cells were assigned as CTCs if there were ≥4 of any of the following morphological characteristics: atypical nucleus (irregular shape or presence of a nodular, lobulated contour); nuclear-cytoplasmic ratio >0.8; nuclear long diameter >18 mm; hyperchromatic nuclei and nonhomogeneous staining; thickened, sunken, wrinkled, and jagged nuclear membrane; presence of nuclear chromatin side-shift or a large nucleoli or presence of abnormal mitotic figures; and presence of tumor cell aggregations, or circulating tumor microemboli.

In the 60 patients, 20 were determined as CTC-positive, a rate of 33.3%.

2.3. Clinical follow-up

The 60 patients with ESCC were followed for at least 2 years. ESCC progression, and times of recurrence, death, and disease-free survival were recorded.

Progression-free survival (PFS) was defined as the time from the onset of CTCs testing to CT progression or patient death. If the patient was lost to follow-up, or at the end of follow-up ESCC had not progressed, then this was recorded as a delete value. When a patient was not rehospitalized after discharge and the telephone follow-up was not answered by patients or their relatives, the patient's initial hospitalization time was also taken as a PFS deletion. Overall survival was analyzed when patients were followed for 3 to 5 years.

2.4. Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences software (SPSS version 22.0). Continuous variables were analyzed by *t* test and expressed as mean ± standard deviation. The chi-squared analysis or Fisher exact test was used to explore any correlation between CTC and clinicopathological variables. Kaplan–Meier survival curves were then used to describe the survival distributions of patients with different levels of CTCs. The log-rank test was used to analyze the survival data and calculate the *P* value. For the multivariate analysis, the Cox regression model was used. The results are presented as hazard ratio (HR) with 95% confidence interval (CI), that is, a multivariate analysis with stepwise regression. Correlations among the number of CTCs, the 2-year PFS, and

Table 1

Clinical pathological characteristics of the patients.

	Surgery + CRT	Surgery alone
Subjects, n	60	50
Age, yr	62.2 ± 7.2 (49.0–78.0)	61.3 ± 6.8 (49.0–78.0)
Gender, male/female	52/8	44/6
Alcohol consumption, yr	12.4 ± 14.8 (0–40)	11.3 ± 14.2 (0–40)
WHO PS, 0/1/2	15/39/6	14/35/1
Tumor location, upper/middle/lower	6/32/22	3/27/20
Tumor size, cm		
≤3	14	11
3–5	28	23
>5	18	16
AJCC-UICC stage, I/II/III/IV	4/13/34/9	4/13/32/1
Differentiation grade, Gx/G1/G2/G3	7/5/27/21	4/5/24/17
Tumor depth, Tis-T1/T2/T3/T4	6/4/26/24	6/4/17/23
LNM, yes/no	36/24	26/24
Lymphatic or venous invasion		
Positive	11	11
Negative	39	39
Unknown	10	0
Serum CEA, ng/mL		
<3.4	45	41
≥3.4	15	9
Platelet × 10^9/L, n*	228.6 ± 74.0 (131–492)	225.0 ± 75.6 (131–492)
N/L ratio*	2.9 ± 1.8 (0.92–8.39)	2.6 ± 1.5 (0.92–8.34)

AJCC-UICC = American Joint Committee on Cancer-Union for International Cancer Control, CRT = chemoradiotherapy, PS = performance status, WHO = World Health Organization.

* Reported a mean (range).

Table 2

Presence of CTCs in the study population of 60 ESCC patients by clinicopathological characteristics.

	CTC$^+$	CTC$^-$	N	P
Subjects, n	20	40		
Age, yr	63.50 ± 6.621	61.60 ± 7.472		.34*
Gender, male/female	19/1	33/7	52/8	.179†
Alcohol consumption, yr‡	13.00 ± 15.93	12.13 ± 14.45*		.832*
Platelet × 10^9/L	254.65 ± 95.426	215.63 ± 57.65*		.053*
N/L ratio‡	3.17 ± 1.55	2.811 ± 1.86*		.462*
Serum CEA, ng/mL				
≥3.4	7	8	15	.206†
<3.4	13	32	45	
WHO performance status, 0/1/2	5/12/3	10/27/3	15/39/6	.65†
AJCC-UICC stage, I/II/III/IV	0/4/11/5	4/9/23/4	4/13/34/9	.26†
AJCC-UICC stage, I-IIIB/IIIC-IV	7/13	28/12	35/25	.01†
Tumor location, upper/middle/lower	2/12/6	4/20/16	6/32/22	.73†
Differentiation, well/mod/poor/other	2/6/11/1	3/21/10/6	5/27/21/7	.10†
Tumor size, cm				
≤3	3	11	14	.512†
3–5	11	17	28	
>5	6	12	18	
Tumor depth, Tis-T1/T2/T3/T4	0/0/9/11	6/4/17/13	6/4/26/24	.082†
LNM, yes/no	12/8	24/16	36/24	1†
Lymphatic or venous invasion				
Positive	4	7	11	.415†
Negative	11	27	38	
Unknown	4	7	11	

* Student *t* test.
† χ^2 test.
‡ Median ± standard deviation.
AJCC-UICC = American Joint Committee on Cancer-Union for International Cancer Control, CTC = circulating tumor cell, PS = performance status, WHO = World Health Organization.

Table 3

Presence of CTCs by clinicopathological characteristics of the 50 surgically treated ESCC patients.

	CTC$^+$	CTC$^-$	N	P
Subjects, n	20	40		
Age, yr	62.20 ± 6.327	60.94 ± 7.100		.557*
Gender, male/female	15/0	29/6	44/6	.16†
Alcohol consumption, yr‡	12.67 ± 15.34	10.71 ± 13.83		.66*
Platelet × 10^9/L	260.87 ± 106.82	209.63 ± 52.20		.026*
N/L ratio‡	2.81573 ± 1.27	2.57 ± 1.62		.612*
Serum CEA, ng/mL				
<3.4	12	29	41	.81§
≥3.4	3	6	9	
WHO PS, 0/1/2	5/9/1	10/25/0	15/34/1	.27§
AJCC-UICC stage, I/II/III/IV	0/4/10/1	4/9/22/0	4/13/32/1	.253§
AJCC-UICC stage, I-IIIB/IIIC-IV	7/8	27/8	34/16	.034§
Tumor location, upper/middle/lower	1/9/5	2/18/15	3/27/20	.82§
Differentiation, well/mod/poor/other	2/5/8/0	3/19/9/4	5/24/17/4	.309§
Tumor size, cm				
≤3	1	10	11	.222§
3–5	9	15	24	
>5	5	10	15	
Tumor depth, Tis-T1/T2/T3/T4	0/0/5/10	6/4/12/13	6/4/17/23	.099§
LNM, yes/no	7/8	19/16	26/24	.621§
Lymphatic or venous invasion, yes/no	4/11	7/28	11/39	.602§

* Student *t* test.
† Fisher exact test.
‡ Median ± standard deviation.
§ χ^2 test.
AJCC-UICC = American Joint Committee on Cancer-Union for International Cancer Control, CTC = circulating tumor cell, ESCC = esophageal squamous cell carcinoma, PS = performance status, WHO = World Health Organization.

TNM staging were analyzed by Spearman method. $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

The sixty patients (53 men, 7 women; 62.2 ± 7.2 years, range 49–78 years) with ESCC were recruited from May to December 2014 (Table 1). All of the patients were being treated for the first time or had experienced a minimum of 6 months without treatment. The 60 patients were treated with chemotherapy, with or without surgery. Among them, a subgroup of 50 patients, who had undergone R0 resection and postoperative adjuvant radiotherapy or chemotherapy (surgery + therapy), were analyzed separately to control for variations in the treatments.

The clinical manifestations of the patients (Table 1) were investigated, including the following: age, gender, duration of alcohol consumption, routine blood analysis, serum carcinoembryonic antigen (CEA) levels, WHO performance status, primary tumor location, tumor size, differentiation, lymph node metastasis, venous invasion, and stage.

3.2. Association between CTC and clinicopathological characteristics

In the total of 60 patients, the presence of CTCs was significantly associated with clinical stages (I-IIIB cf. IIIC-IV $P = .01$) of the cancer (Table 2). CTCs were not significantly associated with patient age, gender, median alcohol consumption, platelet, neutrophil/lymphocyte (N/L) ratio, serum CEA, or WHO performance status. Moreover, CTCs were not correlated with pathological features such as tumor location, tumor size, grade of differentiation, tumor depth, lymph node metastasis, or lymphatic or venous invasion.

In the subgroup of 50 surgically treated patients, the presence of CTCs was significantly associated with platelet ($P = .026$) and clinical stages (I-IIIB cf. IIIC-IV $P = .034$; Table 3). CTCs were not significantly associated with age, gender, duration of alcohol consumption, neutrophil-to-lymphocyte ratio, serum CEA, or WHO performance status. CTCs were also not significantly associated with pathological features such as tumor location, tumor size, grade of differentiation, tumor depth, lymph node metastasis, or lymphatic or venous invasion.

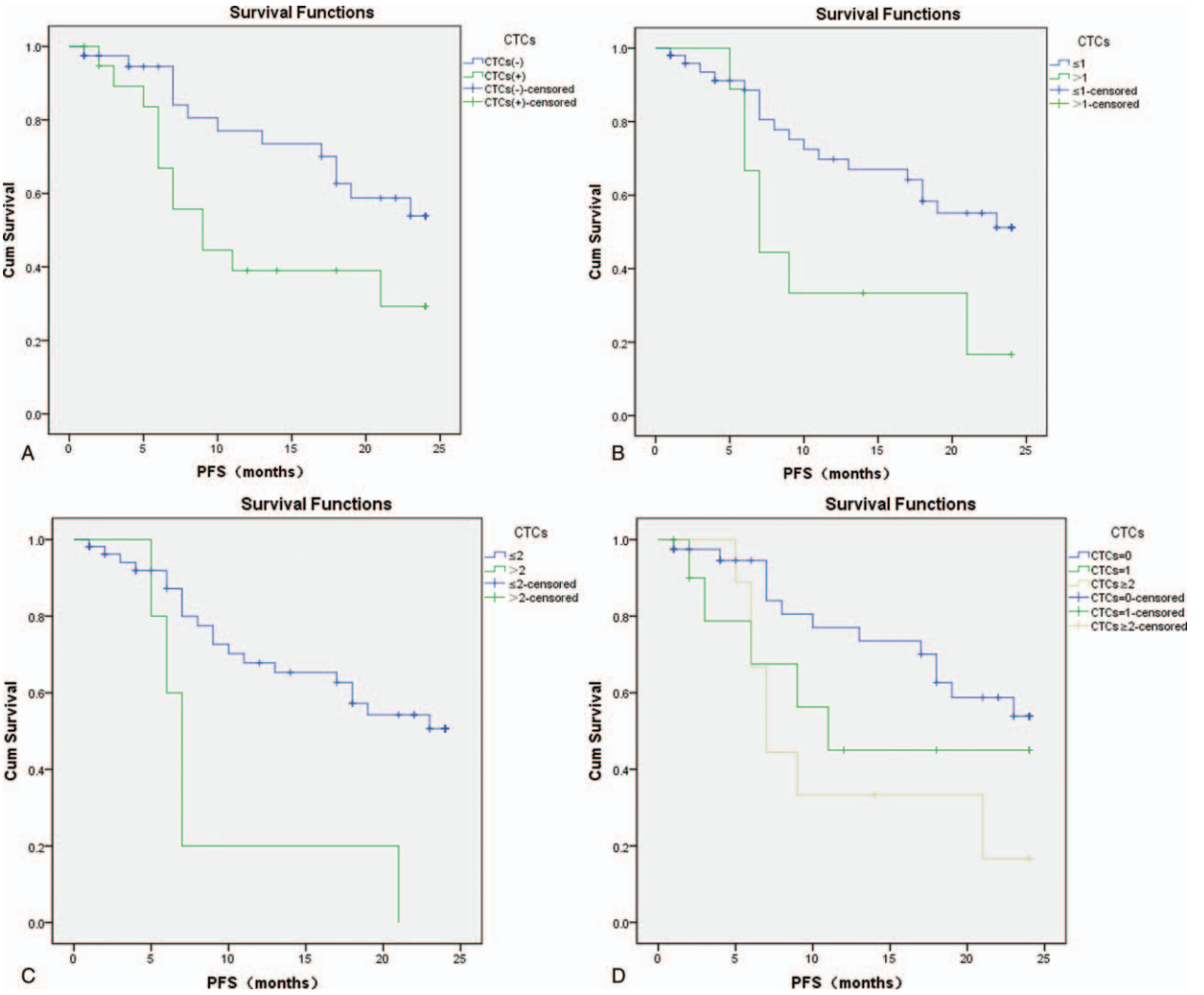

Figure 1. Comparison of the survival time of the patients with different count of CTCs counts in the 60 patient group. (A) Patients with CTCs > 0 cf. CTCs = 0; (B) patients with CTCs > 1 cf. CTCs ≤ 1; (C) patients with CTCs > 2 cf. CTCs ≤ 2; (D), patients with CTCs = 0, CTCs = 1, and CTCs ≥2. CTCs = circulating tumor cells.

3.3. Association between CTC count and survival time

The median survival time of the 60 patients was 21 months. The number of CTCs was associated with PFS (Fig. 1). PFS was significantly shorter for patients with CTCs > 0 compared with patients with CTC = 0; CTCs > 1 compared with CTCs ≤ 1; and CTCs > 2 compared with CTCs ≤ 2 (median survival time: 9 cf. 7 months).

In the group of 50 surgically treated patients, PFS decreased as the CTC count increased ($P = .031$) from 0 to 1, to ≥2 (Fig. 2D).

The 60 patients were further divided into 4 groups according to the presence of CTCs and treatment (Fig. 3A): CTC^+ with surgery; CTC^- with surgery; CTC^+ with chemoradiotherapy; and CTC^- with chemoradiotherapy. The comparative analysis showed that the PFS of the CTC^- with surgery group was significantly longer than that of the other 3 groups ($P = .001$).

The 60 patients were also stratified into 4 groups based on the presence of CTCs and clinical stage (Fig. 3B): CTC^+ and I-IIIB; CTC^- and I-IIIB; CTC^+ and IIIC-IV; and CTC^- and IIIC-IV. The PFS of patients in the CTC^- and I-IIIB group was significantly longer than that of the other 3 groups ($P = .006$).

In the 60 patients, the univariate Cox regression analyses showed that the following were not significantly associated with PFS (Table 4): age, gender, duration of alcohol consumption, platelet, neutrophil-to-lymphocyte ratio, serum CEA, WHO performance status, clinical stage, tumor location, grade of differentiation, tumor size, tumor depth, lymph node metastasis, and lymphatic or venous invasion. Conversely, CTCs > 2 was an independent prognostic marker for PFS ($P = .008$, HR 3.88; 95% CI 1.42–10.56). In addition, the risk of tumor recurrence was 2.3-fold higher in patients in whom CTCs were detected, compared with patients without CTCs ($P = .036$, HR 2.34, 95% CI 1.06–5.17).

The multivariate Cox regression analysis showed that patients with CTCs > 2 had significantly shorter PFS than those patients with CTCs ≤ 2 ($P = .003$, HR 5.63, 95% CI 1.77–17.887). Moreover, tumor location and grade of differentiation were independent prognostic markers for PFS ($P < .05$).

In the 50 surgical patients (Table 5), the univariate ($P = .015$, HR 3.3, 95% CI 1.20–8.65) and multivariate ($P = .022$, HR 10.70, 95% CI 1.40–81.91) analyses showed that the

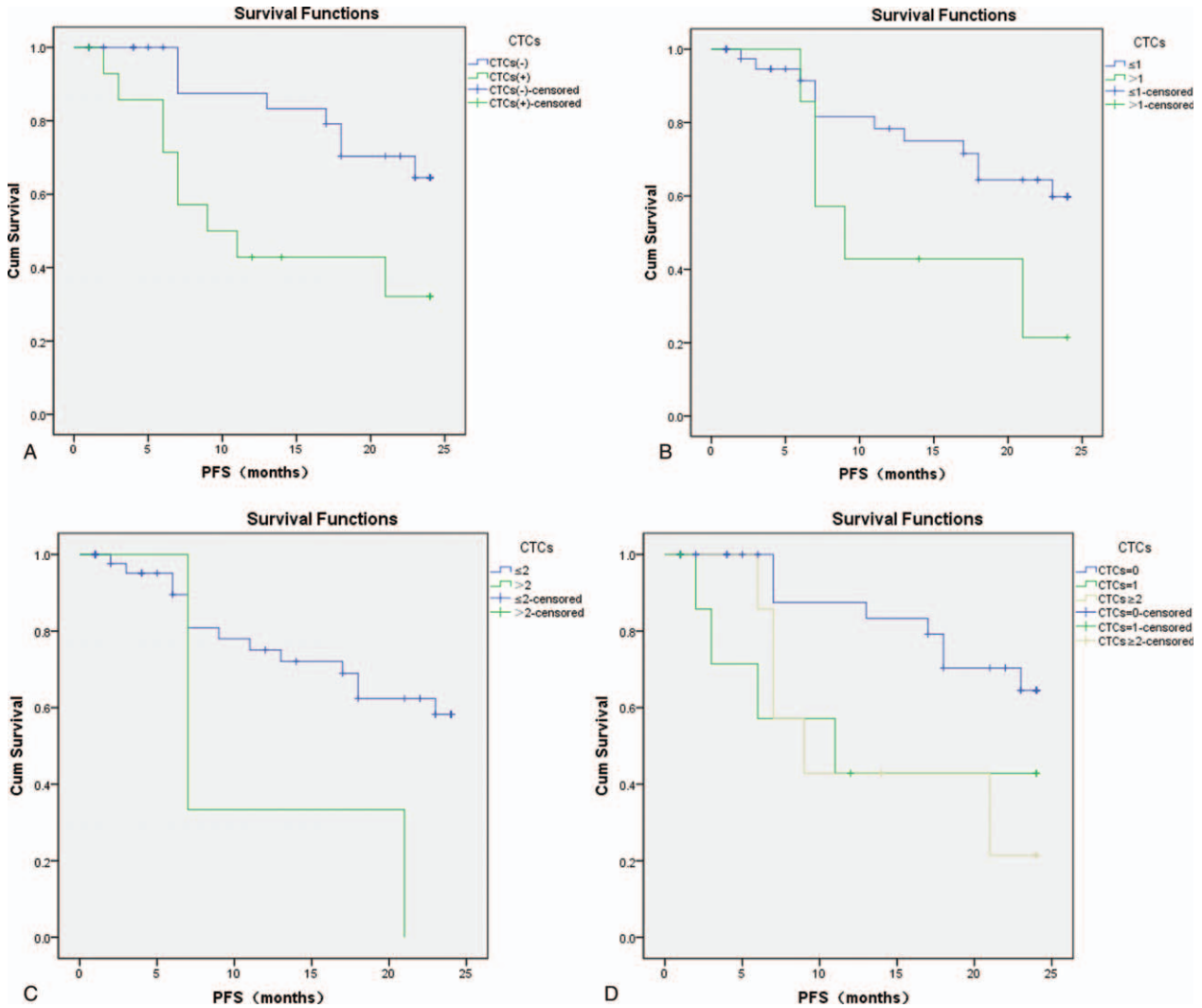

Figure 2. Comparison of the survival time of the patients with different count of CTCs in the 50 patients treated with surgery. (A) Patients with CTCs > 0 cf. CTCs = 0; (B) patients with CTCs > 1 cf. CTCs ≤ 1; (C) patients with CTCs > 2 cf. CTCs ≤ 2; (D), patients with CTCs = 0, CTCs = 1, and CTCs ≥2. CTCs = circulating tumor cells.

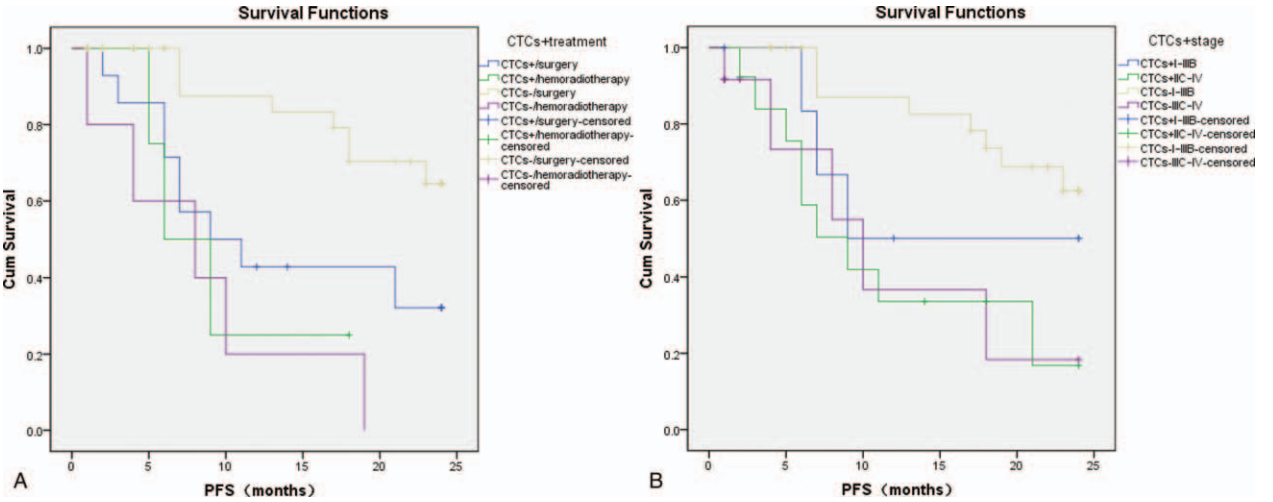

Figure 3. Effect of CTCs on the survival time of the patients when the patients were treated differently or at different stages. (A) Patients treated with surgery cf. chemoradiotherapy; (B) patients at I-IIIB cf. IIIC-IV. CTCs = circulating tumor cells.

preoperative presence of CTCs was significantly associated with a shorter PFS ($P < .05$).

3.4. Association between CTC count and TNM staging

Spearman rank correlation analysis showed that there was a correlation between the number of CTCs and T stage ($r = 0.26$, $P = .043$; Table 6); but no significant correlations were found between the number of CTCs and N stage ($r = -0.037$, $P = .78$; Table 7), or M stage ($r = 0.19$, $P = .15$; Table 8).

3.5. Associations among CTC count, TNM staging, and survival times

Of the 60 patients enrolled in this study, 12 patients were lost. Spearman rank correlation analysis showed that there was a negative correlation between the number of CTCs and PFS in the remaining 48 ESCC patients ($r = -0.342$, $P = .017$; Table 6); but no significant correlation between the number of CTCs and PFS was found when patients were stratified as II/III/IVI-IIIB/IIIC-IV/ I-II/III-IV stages (Table 6).

Table 4

Univariate and multivariate analyses of PFS of 60 patients.

	Univariate			Multivariate		
Prognostic factors	**Hazard ratio**	**95% CI**	***P* value**	**Hazard ratio**	**95% CI**	***P* value**
CTCs (positive vs negative)	2.34	1.059–5.169	.036			
CTCs (≤2 vs >2)	3.876	1.423–10.562	.008	5.627	1.77–17.887	.003
Age (≤65 vs >65)	1.166	0.515–2.638	.713			
Gender (male vs female)	0.724	0.216–2.420	.6			
Alcohol consumption (positive vs negative)	0.682	0.311–1.497	.34			
Serum CEA (≤3.4 vs >3.4)	2.063	0.884–4.813	.094			
Tumor size, cm						
≤3 versus 3–5	0.557	0.219–1.413	.218			
≤3 versus >5	1.121	0.406–3.099	.825			
Tumor location						
Upper versus middle	0.514	0.162–1.631	.259	0.1	0.02–0.493	.005
Upper versus lower	0.54	0.164–1.784	.313	0.043	0.006–0.298	.001
Grade of differentiation						
G1 versus G2	2.024	0.253–16.209	.507	2.595	0.305–22.119	.383
G1 versus G3	5.088	0.666–38.857	.117	8.942	1.005–79.538	.049
G1 versus G4	1.634	0.148–18.027	.689	0.982	0.083–11.640	.989
T stage						
Tis/T1 versus /T2	0.574	0.06–5.523	.63			
Tis/T1 versus /T3	1.093	0.308–3.877	.89			
Tis/T1 versus /T4	1.152	0.312–4.261	.832			
Lymph node metastasis (positive vs negative)	1.779	0.763–4.146	.182			

CI = confidence interval, CTC = circulating tumor cell, PFS = progression free survival.

Table 5

Univariate and multivariate analyses of progress-free survival in the 50 patients.

	Univariate			Multivariate		
Prognostic factors	**Hazard ratio**	**95% CI**	***P* value**	**Hazard ratio**	**95% CI**	***P* value**
CTCs (positive vs negative)	3.3	1.259–8.645	.015	10.697	1.397–81.906	.022
CTCs (≤2 vs >2)	3.388	0.954–12.038	.059			
Age (≤65 vs >65)	0.711	0.232–2.184	.552			
Gender (male vs female)	0.853	0.195–3.736	.833			
Alcohol consumption (positive vs negative)	0.82	0.312–2.156	.687			
Serum CEA (≤3.4 vs >3.4)	1.539	0.499–4.743	.453			
Tumor size, cm						
≤3 versus 3–5	0.56	0.177–1.766	.322			
≤3 versus >5	1.15	0.333–3.977	.825			
Tumor location						
Upper versus middle	0.392	0.079–1.940	.251			
Upper versus lower	0.487	0.098–2.427	.38			
Grade of differentiation						
G1 versus G2	1.565	0.188–13.014	.679			
G1 versus G3	4.386	0.559–34.423	.16			
G1 versus G4	0	0	.982			
T stage						
Tis/T1 versus /T2	0.565	0.059–5.446	.622			
Tis/T1 versus /T3	0.586	0.14–2.455	.465			
Tis/T1 versus /T4	1.06	0.281–4.000	.931			
Lymph node metastasis (positive VS. Negative)	1.174	0.451–3.056	.743			

CI = confidence interval, CTC = circulating tumor cell, HR = hazard ratio.

In the 50 surgically treated patients stratified as stage II or I-IIIB or T3 and T3 + T4, the PFS of the patients with CTCs > 1 or CTCs > 2 was significantly shorter than that of patients with CTCs ≤ 1 or CTCs ≤ 2, respectively ($P < .05$; Tables 7 and 8). In stage III and T3 + T4 groups, the PFS of patients with CTCs > 0 was significantly shorter than that of the patients with CTCs = 0 ($P < .05$).

Table 6

Correlation between the number of CTCs and PFS.

Variable	**S ± D**	**n**	***R***	***P***
All patients				
CTC count	0.75 ± 1.242	48	−0.342	.017
PFS	15.13 ± 7.967	48		
Stage II				
CTC count	0.82 ± 1.537	11	−0.407	.214
PFS	16.09 ± 6.833	11		
Stage III				
CTC count	0.67 ± 1.007	24	−0.366	.079
PFS	17.13 ± 7.703	24		
Stage IV				
CTC count	1.22 ± 1.641	9	0.08	.838
PFS	7 ± 5.123	9		
Stage I-IIIB				
CTC count	0.43 ± 1.073	30	−0.225	.232
PFS	17.83 ± 7.057	30		
Stage IIIC-IV				
CTC count	1.28 ± 1.364	18	−0.032	.899
PFS	10.61 ± 7.484	18		
Stage I-II				
CTC count	0.64 ± 1.393	14	−0.29	.315
PFS	16.29 ± 6.911	14		
Stage III-IV				
CTC count	0.79–1.200	34	−0.336	.052
PFS	14.65–8.413	34		

CTC = circulating tumor cell, PFS = progression free survival.

Table 7

CTC count and PFS by clinical stage.

CTC count	**PFS, mo**	**n**	χ^2	***P***
Stage II				
0	16 ± 4.016	4	0.441	.507
>0	19 ± 2.407	9		
≤1	19.825 ± 2.069	11	6.347	.012
>1	8 ± 1.000	2		
≤2	18.743 ± 2.126	12	4.5	.034
>2	7 ± 0.000	1		
Stage III				
0	13.889 ± 2.814	11	5.206	.023
>0	21.47 ± 1.260	23		
≤1	19.285 ± 1.619	29	1.016	.313
>1	16.1 ± 3.566	5		
≤2	19.032 ± 1.565	32	1.989	.158
>2	14 ± 7.000	2		
Stage IV				
0	8 ± 2.449	5	0.246	.62
>0	5.75 ± 2.016	4		
≤1	7.429 ± 2.039	7	0.531	.466
>1	5.5 ± .500	2		
≤2	7.429 ± 2.039	7	0.531	.466
>2	5.5 ± .500	2		
Stage I-IIIB				
0	15.667 ± 3.421	7	1.396	.237
>0	20.416 ± 1.241	28		
≤1	20.647 ± 1.166	31	5.393	.02
>1	11.5 ± 3.649	4		
≤2	19.85 ± 1.228	34	4.388	.036
>2	7 ± .000	1		
Stage IIIC-IV				
0	11.65 ± 2.341	13	0.006	.94
>0	11.817 ± 3.107	12		
≤1	11.65 ± 2.341	13	0.006	.94
>1	11.817 ± 3.107	12		
≤2	12.332 ± 2.243	21	0.647	.421
>2	9.75 ± 3.772	4		

CTC = circulating tumor cell, PFS = progression free survival.

Table 8

CTC count and PFS in patients by T stage.

CTC count	PFS, mo	n	χ^2	*P*
Stage T3				
0	17.796±1.981	17	1.412	.235
>0	13.175±3.072	9		
≤1	17.464±1.798	23	6.812	.009
>1	7±1.155	3		
≤2	17.044±1.763	24	6.888	.009
>2	6±1.000	2		
Stage T4				
0	20.167±2.565	13	2.676	.102
0	13.1±2.641	11		
≤1	16.75±2.561	18	0.504	.478
>1	14.417±3.345	6		
≤2	16.933±2.284	21	2.087	.149
>2	11.333±4.842	3		
Stage T3+T4				
0	18.684±1.539	30	4.026	.045
>0	13.02±1.996	20		
≤1	17.347±1.450	41	3.819	.051
>1	11.944±2.536	9		
≤2	17.099±1.380	45	7.1	.008
>2	9.2±2.973	5		

CTC = circulating tumor cell, PFS = progression free survival.

4. Discussion

In this study, CTCs were detected in ESCC by using ISET technology, and were found to be associated with the number of platelets, ESCC staging, and patient's PFS. These results indicate that CTCs are independent prognostic indicators of patient clinical outcomes in ESCC.

4.1. CTCs detection in ESCC patients

Methods to detect CTCs in esophageal cancer are mainly RT-PCR, the Celltracks AutoPrep system, and ISET technology. In the present study, among 61 ESCC patients, CTCs were detected in 20 patients via the ISET method, a rate of 32.8%. Whereas, in the same patient cohort as our previous study,[11] CTCs were detected in only 1.6% when tested using the Celltracks AutoPrep system. This strongly indicates that the ISET method is more sensitive than the Celltracks AutoPrep. The greater sensitivity of ISET for detecting CTCs compared with the Celltracks AutoPrep system was also observed by Khoja et al[13] in patients with pancreatic cancer. The difference in the sensitivity of the 2 methods may be because the Celltracks AutoPrep system only detects epithelial CTCs, and misses mesenchymal CTCs.[14]

In other studies, ISET was also found to be a better method for detection of esophageal cancer CTCs compared with RT-PCR or the Celltracks AutoPrep system.[15,16] The high detection of CTCs in ESCC patients may explain the clinical observation that many patients with early-stage ESCC, in whom traditional detection methods found no signs of distant metastasis, soon died of tumor recurrence and metastasis, due to micrometastases from the spread of CTCs.[4]

4.2. CTCs are prognostic of clinical outcomes of ESCC patients

Although the TNM staging system can predict the prognosis of cancer patients and guide clinicians to choose a treatment strategy, combining it with the CTCs count will be more effective.[17,18] The positive association between CTCs count and ESCC clinical stage observed in the present study is in accord with previous observations that CTCs correlated with tumor differentiation, T stage, lymph node micrometastasis, and pathological stage.[19,20]

Our observation of an association between CTCs and the number of platelets is in agreement with the finding that platelets facilitate the generation of CTCs, and protect them from various host attacks, such as immune assaults, apoptosis, and shear stress.[21] Platelets also regulate the intravasation/extravasation of CTCs, and promote the survival of CTCs in the blood-stream.[22] The negative correlation found between PFS and CTCs in the ESCC patients of the present study was also found in patients with breast, pancreatic, or prostate cancer.[22–24] All these results indicate that CTCs are prognostic indicators of disease progress and poor clinical outcomes in ESCC patients.

5. Conclusion

This study is the first to show that the CTC, detected by ISET, is an independent and prognostic indicator of patients' outcome in ESCC. CTCs in patients with ESCC may lead to micrometastases that cannot be detected by traditional examination methods. CTCs detected by ISET technology may be used as prognostic indicators of disease progress and clinical outcomes in ESCC.

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