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REVIEW

Circulating Tumor Cells in Esophageal Squamous Cell Carcinoma – Mini Review

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Abstract: Esophageal cancer has high incidence and mortality rates and a low five-year survival rate of <15% owing to its strong capabilities of invasion, relapse and metastasis. The classic view holds that metastasis and diffusion is an advanced event during cancer progression, but recent studies show that distant diffusion of primary cancer cells may actually be an early event. Detection of circulating tumor cells (CTCs) in the circulation may indicate tumor spread, so CTCs are considered to be the key factor of metastatic cascade. In recent years, despite research progress on CTCs, there is a lack of systematic and important evidence to confirm the diagnostic, monitoring and prognostic values of CTCs in esophageal squamous cell carcinoma (ESCC). In this review, we clarify the relationship between CTC values and ESCC and provide more reliable evidence to improve the management and treatment of ESCC.

Keywords: esophageal squamous cell carcinoma, circulating tumor cells, diagnostic, prognostic

Introduction

The global incidence of esophageal cancer (EC) is increasing. EC is the seventh most common cancer worldwide and its mortality rate ranks the sixth.[1] The two major histologic subtypes of EC are squamous cell carcinoma and adenocarcinoma. The former is more prevalent in East Asia, East and South Africa, and South Europe, whereas the latter is much more common in North America and other parts of Europe.[2] In Asian countries, esophageal squamous cell carcinoma (ESCC) accounts for >90% of all cases of EC.[3]

EC patients at early stage have no typical symptoms or disease-related symptoms, so most EC patients are found with advanced disease that is often incurable or directly invades into adjacent organs and distant metastases.[4,5] Radical resection of EC is still the main treatment, but because of high recurrence and mortality rates,[6] postoperative symptoms such as appetite loss, early satiety and dysphagia can impair the quality of life of patients.[7] Neoadjuvant chemoradiotherapy (CRT) followed by surgery or concurrent CRT (CCRT) is a standard approach for treatment of localized ECs and can improve overall survival compared to esophagectomy alone.[8] However, although EC treatment has advanced greatly in recent decades, the treatment outcomes are still poor, and the five-year survival rate is less than 15%.[09–13] The poor prognosis is largely due to the rapid progress of local recurrences and metastasis. Thus, tumor markers that can clarify the treatment response and early tumor recurrence/metastasis are needed. At present, the commonly-used tumor markers in clinic are serum

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biomarkers (eg carcinoembryonic antigen (CEA), SCC antigen, and cytokeratin 19 fragment), which have low sensitivity and specificity for early diagnosis or recurrence,[14] however. Therefore, highly sensitive and specific biomarkers are urgently needed in clinical practice. There is an unmet clinical need to identify biomarkers that can sensitively detect residual disease and/or early progression in EC patients.[15–17] Hence, useful non-invasive biomarkers in blood samples are considered to be valuable and convenient for the early detection and subsequent management of cancers.[18]

Circulating tumor cells (CTC) come from tumor cells that escape from the primary tumor, then circulate in the vascular system, and extravasate into distant organs to form metastases (Figure 1).[19] CTCs were first described by Thomas Ashworth in 1869.[20] The significance of CTCs in peripheral blood is extensively studied in various malignancies.[21–25] The CTCs in peripheral blood are potentially correlated with tumor metastasis/recurrence of breast,[21,22] prostate,[23] lung,[24] and colon rectal cancers.[25] As the detection is relatively convenient, CTCs as liquid biopsies are an emerging noninvasive tool for cancer diagnosis, surveillance and treatment. Multiple studies prove the important diagnostic, prognostic and therapeutic implications of CTC monitoring.[26–31] Nevertheless, systematic and significant evidence that validates the diagnostic, monitoring and prognostic values of CTCs in ESCC is lacking. In this review, we clarify the relationship between CTCs and the values of ESCC patients, and provide more reliable evidence that may improve the management and treatment of ESCC.

Circulating Tumor Cells Capture

CTCs are released from primary tumors or metastatic sites into the bloodstream.[20] Most exfoliated tumor cells may die in the circulatory system due to physical and anatomical conditions, but some residual CTCs with particular malignant potential acquire stem cell characteristics and eventually develop metastatic tumors.[32] Hence, CTCs exist as rare cells in the blood (one CTC in 10^6–10^9 blood cells).[33] Isolation and subsequent quantitative and qualitative analysis across different stages of the disease prove the prognostic and predictive significance of CTCs in different malignancies.[34] Methods to detect CTCs mainly include reverse transcription polymerase chain reaction (RT)-PCR, the CellSearch® detection system, isolation by size of epithelial tumor cells (ISET),[35] and fluid-assisted separation technique (FAST).[36] PCR-based methods are widely applied in CTC detection, but are mainly limited by the inability in visualization, enumeration or evaluation of viability of CTCs, which are destroyed during RNA isolation.[37] The CellSearch® system developed and approved by the US Food and Drug Administration in 2004 still has no standard method or protocol for identification or isolation of CTCs because of the relatively low detection efficiency.[3,38–41] Studies show that CTCs during metastasis often experience epithelial mesenchymal transformation, including the loss of epithelial markers and transformation into interstitial components, leading to the escape of cells from the detection system based on epithelial markers.[42] Therefore, in clinical practice, the detection technology should be improved to avoid this disadvantage.

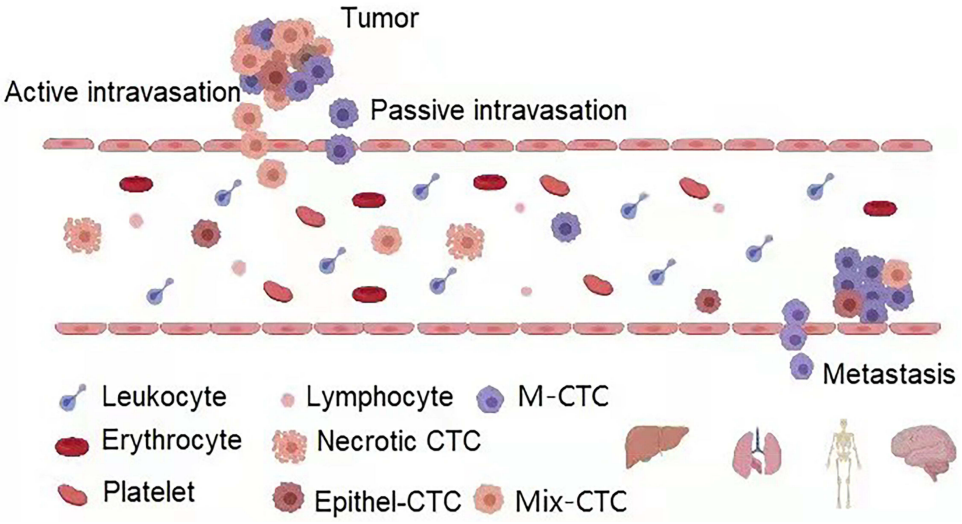

Figure 1 Production and metastasis of circulating tumor cells.

ISET with higher sensitivity and specificity assesses differences in the diameters of tumors and normal blood cells, and separates CTCs and/or circulating tumor microemboli from normal blood cells with a membrane filter. FAST is also recognized in clinical practice.[3,35,39] It proves FAST is capable of highly sensitive, selective, rapid and label-free isolation of CTCs from the whole blood directly without prior sample manipulation.[36,43,44]

Circulating Tumor Cells in ESCC Diagnosis

Most ESCC patients experiencing poor outcomes are mainly due to delayed diagnosis as a result of late presentation of symptoms or structural changes. Specific clinical symptoms and signs are usually unhelpful in making early diagnosis and results of most diagnostic studies are unreliable. Moreover, widespread screening is usually impossible.[45] CTCs isolated from the peripheral blood of primary tumors are increasingly studied owing to their prognostic value in many tumors and are recognized as a key factor in tumor metastasis.[46,47] However, there are few studies on CTCs in the early diagnosis of EC, because the CTCs are rarely or even not detected in healthy controls or benign disease.[3,16,36,48–53]

Li et al[3] detected the CTC values in peripheral blood of 61 ESCC patients and 22 normal control subjects using CellSearch and ISET, and compared the sensitivity and specificity of the two methods. However, neither method detected CTCs in the healthy controls. Similarly, Qiao et al[52] studied 103 peripheral blood samples from 59 ESCC patients and 25 healthy subjects, evaluated the CTC diagnostic value and optimal CTC cut-off level of overall survival (OS) and relapse-free survival (RFS) in ESCC patients. The CTC count was>3 in 24 patients (54.5%) and >5 in 14 patients (31.8%), but no CTCs were found in the blood samples of healthy subjects. Also Su et al[50] studied the changes of CTCs before and after treatment and the relationship of CTCs with prognosis in 75 ESCC patients treated CCRT. It was concluded similarly that the CTC numbers of 57 EC patients were significantly higher than those of 20 healthy donors. Allard et al[16] used a CellSearch® system to detect the value of peripheral blood CTC in 199 patients with benign diseases, 964 patients with metastatic cancers, and 145 healthy controls. Of the 344 healthy and non-malignant subjects, only one subject (0.3%) had > 2 CTCs per 7.5 mL of blood. In 2183 blood samples from 964 patients, the range of CTCs in patients with metastatic cancers was 0 to 23,618 CTC per 7.5 mL (mean 60 ± 693 CTC per 7.5 mL), and 36% (781 of 2183 samples) had more than 2 CTCs.

Due to the limitations of CTC detection technology, the detection rate of CTCs is often low. In clinic, special markers detected by PCR represent CTC, and related studies are conducted, bringing similar conclusions. Kaganoi et al[49] detected the peripheral blood CTCs (CEA and *SCCA* mRNA) of 70 EC patients, and showed that 23 patients (33%) were positive for *SCCA* mRNA on admission, but *SCCA* mRNA expression was undetected in blood samples from either healthy volunteers or patients with benign disease. Andolfo et al[51] studied the changes of serum CTC (copy-number variations of erbB2) in 41 ESCC patients and 34 healthy volunteers, and found that 24 ESCC patients had copy-number variations of erbB2 (CN) ≤2 (58.5%), while 17 ESCC patients had CN >2 (41.4%) with a median CN of 2 ± 5.02, but the 34 healthy control subjects showed a median erbB2 CN of 1± 0.16. Similarly, Liu et al[48] discussed the serum levels of CEA mRNA gene expression in 53 EC patients before surgery, immediately after surgery, and the 3rd day postoperatively quantified by PCR, and detected the changes of CEA mRNA before and after surgery, and the prognosis of patients with preoperative and postoperative positive CEA mRNA in comparison with 22 cases of benign esophageal tumors and 30 healthy controls. It was found the cells expressing CEA mRNA were below the detection limit in 22 benign patients or 30 healthy volunteers at all three time points. Different CTC detection techniques draw the same conclusion. Choi et al[36] detected CTCs in 73 ESCC cases and 31 healthy controls by FAST, and found CTCs in 3 healthy controls (9.6%) and 63 ESCC patients (86.3%), and the 63 ESCC patients had CTC ≥ 2 CTC per 7.5 mL of blood. Based on the above studies, we have reason to believe that the serum CTC contents in benign tumors and normal people are extremely low or undetectable. Therefore, when CTCs are detected, the possibility of tumor is often considered. CTC detecting can be considered before endoscopic examination for patients with suspected esophageal cancer. Combination of the two may improve diagnosis rates and even clarify tumor load (Table 1).

Circulating Tumor Cells in ESCC Surveillance

In the treatment of EC, evaluation of treatment effect and monitoring of tumor status are important. Traditionally,

Table 1 Circulating Tumor Cells in Esophageal Squamous Cell Carcinoma Diagnosis

Author	Country	Year	Number of Patients	Detect Technology	CTC Type	Main Findings
Li et al[3]	China	2015	61 ESCC and 22 healthy volunteers	Cell-search and ISET	CTC	Cell-search detect CTC1.6% (1/61), no CTM detected ISET detect CTC 32.8% (20/61), detect CTM 4.9%(3/61) healthy volunteers: Cell-search and ISET(0/22)
Kaganoi et al[49]	Japan	2004	70EC,19 healthy volunteers and 3 benign tumour	RT-PCR	SCCA mRNA	SCCA mRNA not detected in healthy volunteers or benign disease 23/70 (33%) patients were positive for SCCA mRNA on admission
Su et al[50]	China	2016	57 ESCC and 20 healthy volunteers	Cell-search	CTC	CTC in 57 EC was higher than 20 healthy blood donors (P=0.04)
Andolfo et al[51]	Italy	2011	41ESCC and 34healthy volunteers	RT-PCR	erbB2	erbB2 CNs were higher than 34 healthy volunteers (P = 0.001)
Liu et al[48]	China	2007	53 EC,22 benign tumour and 30 healthy volunteers.	RT-PCR	CEA mRNA	CEA mRNA-positive: B-1:188(95% CI,155–498) B0:1513 (95% CI,660–7974) B+3:707(95% CI,737–3005) CEA mRNA were lower than the detection limit in 22 benign tumour and 30 healthy volunteers.
Tanaka et al[53]	Japan	2010	244 ESCC and 20 healthy volunteers	RT-PCR	CEA mRNA and SCC mRNA	Pre-CTC(+):34 patients (13.9%) Pro-CTC(+):41 patients (16.8%) healthy volunteers (0/20)
Qiao et al[52]	China	2017	59ESCC and 25healthy volunteers	Cell-search	CTC	Pre-treatment CTC(+):47/59 (79.7%) healthy volunteers (0/25)
Choi et al[36]	Korea	2019	73ESCC and 31healthy volunteers	FAST	CTC	ESCC:CTC(+)63/73 (86.3%) healthy volunteers:3/31 (9.6%)

Abbreviations: CTC, circulating tumor cells; ISET, isolation by size of epithelial tumor cells; (RT)-PCR, reverse transcription polymerase chain reaction; CTM, circulating tumor microemboli; ESCC, esophageal squamous cell carcinoma; FAST, fluid-assisted separation technique.

clinical evaluations and imaging (eg endoscopy, endoscopic ultrasonography, computed tomography, magnetic resonance imaging, even PET-CT) are insufficient for independent evaluation of treatment effect.[54,55] With CTC monitoring before and after treatment, multiple prospective studies show that the changes of CTCs before and after treatment are related to tumor stage, lymph node metastasis status and hematogenous metastasis, and moderately reflect tumor status.[26,50,53,56–58] These studies prove that CTCs as independent predictors of metastasis and recurrence may allow better stratification of patients than classic parameters (eg TNM classification) and imaging methods.

Yin et al[26] detected the positive rates of peripheral blood CTCs (CEA, CK19 and Survivin) of 72 ESCC patients before and after radiotherapy using PCR, and found poor radiotherapy efficacy was significantly correlated with CTC (+) pro-radiotherapy, but not with CTC(+) pre-radiotherapy. In addition, the role of survivin in monitoring ESCC was also studied. Cao et al[58] explored the serum expressions of circulating cancer cells (CCCs) (survivin mRNA) in 108 ESCC patients before and after treatment by using PCR. It was found the survivin expression of CCCs was a significant risk factor only for metastasis, and thus may be an indicator of metastasis in ESCC patients. Moreover, the cumulative recurrence rate of survivin-positive patients was significantly higher than that of negative patients. Besides, Tanaka et al[53] investigated the changes of CTCs (CEA mRNA and SCC mRNA) in EC patients before and after operation by PCR, and found that CTC positive patients after chest surgery had significantly more metastatic lymph nodes and higher degree of lymph infiltration.

Similar research indicates that CTCs with markers of tumor initiating cells or cancer stem cells are responsible

for recurrence and metastasis. Nakashima et al[57] also detected the changes of serum CTCs (CEA mRNA) in 50 ESCC patients after anesthesia, preoperatively and postoperatively by PCR, and obtained similar results. They illustrated the incidence of lymph node metastasis in the CTC(+) expression group was significantly higher than that in the non-expression group, and the positive rate significantly increased with the increment of tumor stage. Therefore, researchers believe that the value of CTC detection is greater at the later tumor stage. Yet, the recurrence rate of CTC(+) patients was significantly higher than that of CTC(-) patients. A case study was conducted by Qiao et al[56] to understand the changes of CTCs in pre- and post-chemotherapy, pre- and pro-radiotherapy, recurrence, and disease stability for ESCC. It was found the CTC changes were consistent with imaging results. Furthermore, the postoperative CTC increased, and CTC remained at a high level after radiotherapy and chemotherapy, the number of CTCs decreased after comprehensive treatment, the CTC monitored for many times was 0, and the tumor condition was stable. Finally, it was confirmed positive postoperative CTC was associated with poor prognosis of patients.

The effects of positive CTC after treatment were identified, so what is the impact of routine pre-treatment CTC test on the formulation of follow-up treatment plan? Matsushita et al[59] suggested that early detection of CTCs may provide important information for treatment, including surgery, chemotherapy, and CRT. Neoadjuvant therapy may be effective in the case of pro-surgery CTC(+). Moreover, the pro-surgery CTC(+) rate in patients receiving chemotherapy alone was significantly higher in comparison with CCRT. Zhao et al[60] monitored preoperative and postoperative CTCs of 115 ESCC patients, and found that CTC(-) patients upon admission were not significantly different in 2-year PFS or OS between the preoperative chemotherapy group and the non-chemotherapy group (PFS: 53.33% vs 58.06%; OS: 38.88% vs 66.63%). But for patients with CTC(+) upon admission, the 2-year PFS of patients receiving preoperative chemotherapy was significantly better than that of patients not receiving preoperative chemotherapy (71.90% vs 38.73%). Besides, Klein et al[61] showed that the detection of serum CTCs in patients with early-stage cancer often indicates the occurrence of hematogenous spread prior to lymph node metastasis. Therefore, monitoring the changes of CTCs in peripheral blood of ESCC patients can dynamically understand the tumor changes and development in patients in real time, so as to develop the appropriate treatment plan and achieve a real sense of personalized treatment (Table 2).

Circulating Tumor Cells in ESCC Prognostic Value

The impact of CTC presence in the peripheral blood of ESCC patients on prognostic value is already evaluated.[26,33,35,36,53] In fact, studies also show that the prognosis of CTC(+) patients is worse than that of CTC(-) patients. Yin et al[26] confirmed the 2-year PFS of CTC(+) ESCC patients before or after radiotherapy was significantly lower than that of CTC(-) patients (mean 18.3 months (95% confidence interval [CI]: 16.7–19.9) vs 21.5 months (19.5–23.6), mean 16.3 months (14.4–18.2) vs 22.8 months (21.8–23.8). Han et al[35] collected peripheral blood from 60 primary EC patients before treatment, and captured CTC ISET, with a CTC(+) ratio of 33.3%. Results showed that CTC(+) significantly shortened PFS than CTC(-) did. PFS was negatively correlated 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% CI 1.77–17.89). Subgroup analysis of 50 patients undergoing R0 resection and postoperative adjuvant radiotherapy or chemotherapy demonstrated CTC was a strong independent prognostic indicator of tumor recurrence (HR10.70; 95% CI, 1.40–8 1.91). Similarly, Reeh et al[33] evaluated 100 patients with EC peripheral blood CTC values by a CellSearch® system, and found the overall CTC detection rate was 18.0%, and the CTC counts ranged from 1 to 56 cells/7.5mL blood. Furthermore, CTC detection may be a stronger indicator of OS and RFS than pathological LN stage. The risk of tumor recurrence was 5.1 times significantly higher if CTCs were detected (HR, 5.063; 95% CI, 2.233–11.480). Patients with CTCs did significantly suffer from worse OS and RFS compared with patients without CTCs, and CTC(+) patients had significantly worse OS and RFS than patients with pN+, M0, CTC(-). As for LN-negative patients, CTC detection showed significant prognostic impact on OS and RFS. Tanaka et al[53] also confirmed that disease-free survival of CTC(-) patients after chest surgery was significantly better than CTC(+) patients. Multivariate analysis found that postoperative CTC status was a significant independent prognostic factor for EC (HR=1.647; 95% CI, 1.032–2.629). But there was no significant difference in OS between patients with preoperative CTC (+) and CTC(-).

Table 2 Surveillance Value of Circulating Tumor Cells in the Treatment of Esophageal Squamous Cell Carcinoma

Author	Country	Year	Research Type	Number of Patients	Detect Technology	CTC Type	Main Findings
Yin et al[26]	China	2012	Prospective	72 ESCC	RT-PCR	CEA, CK19, survivin	Pro-RT CTC(+) was correlated with poor radiotherapy efficacy (P=0.027), pre-RT CTC(+) was not correlated with radiotherapy efficacy (P=0.846).
Cao et al[58]	China	2009	Prospective	108 ESCC	RT-PCR +ELASA	Survivin mRNA	1.CCC(+) &CCC(-): higher recurrence rate (P = 0.002) CCC(+): initial and follow up significantly higher than CCC (-) (P = 0.021, Chi-square test)
Nakashima et al[57]	Japan	2003	Prospective	50 ESCC	RT-PCR	CEA mRNA	31 patients (57.4%) were positive for CTC; CTC (+) &CTC (-): more lymph node metastasis(*P* = 0.011); CTC (+): later stage (*P*=0.048)
Qiao et al[56]	China	2015	Case report	1ESCC	Negative enrichment method	CTC	CTC changes were consistent with imaging results; Pro-treatment CTC count transient increased; The tumor stable, CTC not detect
Zhao et al[60]	China	2020	Prospective	117ESCC (57: preoperative chemotherapy group and 58 surgery ± chemotherapy group)	Negative enrichment methods	CTC	First time: Pre-group CTCs(+):49.12% (28/57) Post-group:55.17%(32/58); Postoperative: Pre-group CTCs (+):52.63% (30/ 57) Post-group:56.90% (33/58); First time CTC (+): preoperative chemotherapy had a better 2-year PFS than CTC(-)(P = 0.037); First time CTC (-): preoperative chemotherapy &not 2-year PFS no significant difference(P = 0.5)
Tanaka et al[53]	Japan	2010	Prospective	244 ESCC	RT-PCR	CEA mRNA and SCC mRNA	Pro-treatment CTC(+): more metastatic lymph nodes (P = 0.002) and higher degree of lymph infiltration (P = 0.008).
Matsushita et al[59]	Japan	2015	Prospective	90ESCC	Cell-search	CTC	Pre-treatment CTC(+): 27.8%(25/90); CTCs(+): related with distant metastasis (p = 0.002); CTC (+): related with pleural dissemination or hematogenous metastases (p<0.0001, p = 0.015)

Abbreviations: CTCs, circulating tumor cells; ESCC, esophageal squamous cell carcinoma; RT, radiotherapy; RT-PCR, reverse transcription- polymerase chain reaction.

However, studies also showed that CTC(+) before treatment also affected OS and PFS. Qiao et al[52] confirmed the OS and PFS of patients with CTC counts >3 or >5/7.5 mL of peripheral blood before surgery were significantly shorter than those of patients with CTC counts<3 or<5/7.5 mL. Su et al[50] showed that the number of CTCs pre-CCRT can be used as a significant prognostic factor for disease-specific PFS and OS in advanced ESCC patients. The number of CTC pre-CCRT showed an independent prognostic effect on disease-specific PFS and OS (HR (95% CI, 3.113 (1.427–6.791) and 1.002 (1.000–1.004), respectively).

Similar results indicating the prognostic significance of CTCs in EC patients were already published in meta-

analyses. Qiao et al[44] proved CTCs were significantly associated with poor OS (HR (95% CI) =1.71 (1.30, 2.12)) and PFS (1.67 (1.19, 2.15)) in 1260 EC patients. Subgroup analysis indicated that presence of CTCs was closely associated with worse OS (Asian: HR (95% CI) =1.66 (1.24–2.08), SCC: 1.66 (1.24–2.08)) and PFS (Asian: 1.63 (1.15–2.12), SCC: 1.63 (1.15–2.12)).

As for the effect of CTC on ESCC, a similar situation exists in EAC. Sclafani et al[62] included obtained the peripheral blood CTC from 22 cases of locally advanced or metastatic gastroesophageal junction adenocarcinoma before and after chemotherapy and at the time of progression through CellSearch system, and clarified the changes and significance of serum CTC before and after treatment.The number of CTCs detected during chemotherapy decreased in all patients with baseline CTCs>2, which reflects the response to chemotherapy to some extent. Overall median progression-free survival was 5.5 months, and was 6.1 months in the patients with < 2 CTCs and 5.2 months in the patients with >2 CTCs (HR 1.06; 95% CI, 0.37–3.03). Median OS was 8.3 months and was 10.5 months in the patients with < 2 CTCs and 6.1 months in the patients with >2 CTCs (HR, 0.52; 95% CI, 0.18–1.50; p=0.23). Similar results were found in studies of other tumors. Tsai et al[63] detected CTCs in the two groups of breast cancer xenograft mice at different growth time points, and found the proportion of CTC detection increased with tumor growth. Furthermore, the CTC number, tumor size, and vascular density all increased significantly with the time of tumor progression, while the correlation of CTCs to vascular density was more significant than to tumor size. Thus, we think noninvasive monitoring of CTC changes is of great significance for clinical practice (Table 3).

The above studies show that with continuous growth of tumors, the CTCs in peripheral blood increase continuously, and the the value of CTCs is greater and the prognosis is worse at higher tumor stage. Therefore, routine detection of CTCs during treatment and at the end of treatment can be used to clarify the tumor situation. Studies also show that changes in CTCs before and after treatment partly reflect tumor response to treatment, so relapse or metastasis can be identified by monitoring the dynamic changes of CTCs.

Circulating Tumor Cells Cutoff Values in ESCC

Due to the limitations of the existing CTCs detection methods and the extremely low content of CTCs in circulating blood, the detection rate of CTC is low. The cutoff value for the clinical impact of CTCs may vary among different detection methods. In fact, different methods produce different recovery and purity rates.[50] Noticeably, the numbers of CTCs obtained by different methods should not be compared for clinical significance. In addition, the cutoff values differ among CTCs detection methods. Hence, we need to know how much value of CTC in serum is meaningful.

Su et al[50] used CD45+ cell removal and positive selective flow cytometry for EpCAM and cytokeratin to detect CTCs and found CTCs ≥21/mL played independent prognostic roles. Andolfo et al[51] conformed that CTC (erbB2 CN) >2 was significantly negatively correlated with survival rate in EC patients by real-time PCR. Han et al[35] showed that CTCs>2 was an independent prognostic marker for PFS (HR 3.88; 95% CI 1.42–10.56) based on ISET. Multivariate analysis by Qiao et al[52] showed that peripheral blood CTCs >5/7.5 mL was a strong prognostic indicator of OS (HR 12.478; 95% CI, 8.2–34.3) and PFS (HR 6.524; 95% CI, 1.2–34.3). The CTCs were detected by CellSearch system. With FAST and a threshold of CTCs≥2/7.5 mL of blood, Choi et al[36] found the sensitivity and specificity to differentiate ESCC patients from healthy controls were 86.3% and 90.3%, respectively. Lee et al[64] used FAST to detect CTCs in ESCC patients and the expression of CTCs with positive TWIST, and also set the cut-off CTCs≥2/7.5 mL of blood. Choi et al[36] evaluated the CTCs in peripheral blood of 63 ESCC patients and 28 healthy controls before treatment by FAST, and clarified the role of CTCs in early diagnosis of EC according to the distribution of CTC value in the two groups. It was also confirmed the CTCs cut-off value of≥2/7.5mL blood will help to distinguish ESCC patients from healthy controls (Table 4).

Clinical Use Limitations of Circulating Tumor Cells

Circulating tumor cells (CTCs) can dynamically monitor the tumor state. However, the clinical application of CTCs shall be focused on five aspects: high heterogeneity and defects with epithelial mesenchymal transformation (EMT), low detection rate, low specificity, large individual difference, and high cost, which limit the wide use of CTCs in clinic. In addition, cell-free DNA (cfDNA) can originate directly from the viable tumor cells or from CTCs by apoptosis, necrosis, autophagy, microenvironmental stress, mitotic catastrophe, trauma, and

Table 3 Prognostic Value of Circulating Tumor Cells in Esophageal Squamous Cell Carcinoma

Author	Number of Patients	CTC Positivity	Main Findings
Yin et al[26]	72 ESCC and 10ESCC tumor tissues	Pre-radiotherapy :54.2% (39/72) Post-radiotherapy :38.9% (28/72)	2 year PFS18.3 months in pre-RT CTC(+) vs median 21.5 months in pre-RT CTC(-); p= 0.01; 2 year PFS 16.3 months in pro-RT CTC(+) vs median 22.8 months in pro-RT CTC(-); p< 0.001; pre-RT CTC(-) and pro-RT CTC(-):CR + PR 73.7%(14/19).
Han et al[35]	60ESCC	33.3%(20/60)	PFS for CTC(-) longer than other group, p=0.001 HR=3.88 for PFS, p=0.008; HR=2.34 for tumor recurrence CTC (+)was 2.5 times vs CTC (-); P=0.036; Shorter PFS in patients with CTC>2/mL vs CTC≤2/mL; p< 0.003
Reeh et al[33]	100EC	18% (18/100)	Shorter OS and RFS in patients with CTC(+) vs CTC(-); p= 0.007, p<0.001; HR=5.06 for tumor recurrence CTC (+)was 5.1 times higher than that of CTC (-);P<0.001;
Tanaka et al[53]	244 ESCC	Pre-surgery:13.9% (34/244) Post-surgery:16.8% (41/144)	Shorter DFS with CTC(+) vs CTC(-); p=0.038; Multivariate analysis: post-operative CTC status was an independent prognostic factor (HR, 1.647; 95% confidence interval, 1.032–2.629; p = 0.03);
Qiao et al[52]	59 ESCC	79.7% (47/59)	Shorter OS and PFS for patients with CTCs > 3 vs CTCs < 3 (mean time; OS was 447 days vs 889 days, PFS was 419 days vs 859 days, p= 0.002); CTCs> 5 worse OS and PFS (mean time; OS was 385 days vs 911 days, PFS was 348 days vs 875 days, P, 0.001); HR=12.5 for OS p<0.05; with CTC (+) vs CTC(-); HR =6.5 for PFS p<0.05; with CTC (+) vs CTC(-); CTC detected about 50.0% (4/8) in the early stage, 84.3% (43/51) in the late stage (p= 0.025).
Su et al[50]	57 ESCC	44.6± 9.1%	HR=3.13 for disease- specific PFS p=0.04; with CTC(+) patients vs CTCs(-); HR= 1.002 for OS p=0.02); with CTC(+) patients vs CTCs (-); multivariate analysis for disease- specific PFS and OS, the CTC number (≥21.0 cells/mL) be independent prognostic factors (p=0.004, p=0.028);
Qiao et al[44]	1260 EC		HR =1.71 for OS, p<0.001; with CTC(+) patients vs CTCs(-); HR =1.67 for PFS, p<0.001; with CTC(+) patients vs CTCs(-); Subgroup analysis: HR =1.66 for OS, p<0.001; HR =1.63 for PFS p<0.001.

Abbreviations: CTCs, circulating tumor cells; ESCC, esophageal squamous cell carcinoma; CCRT, concurrent chemoradiotherapy; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; DFS, disease-free survival; RFS, relapse-free survival.

Table 4 Cut off Value of CTCs with Different Detection Techniques

Author	CTCs Detect Technology	CTCs Cutoff Value
Su et al[50]	Cellsearch	≥21/mL blood
Andolfo et al[51]	RT-PCR	>2/7.5mL blood
Han et al[35]	ISET	>2/7.5mL blood
Choi et al[36]	FAST	≥2/7.5 mL blood
Lee et al[64]	FAST	≥2/7.5 mL blood
Qiao et al[52]	Cell Search	≥5/7.5 mL blood
Choi et al[36]	FAST	≥2/7.5 mL blood

Abbreviations: (RT)-PCR, reverse transcription polymerase chain reaction; ISET, isolation by size of epithelial tumor cells; FAST, fluid-assisted separation technique; CTCs, circulating tumor cells;

treatment procedure.[65] CfDNA retains some properties of nuclear chromatin during DNA release.[66] The cfDNA of tumor patients contains various somatic mutations the same as tumor gene mutations, such as oncogene and tumor suppressor gene mutations, microsatellite changes, promoter methylation and loss of heterozygosity. Therefore, cfDNA has high specificity and becomes a hit in the research of many medical disciplines.[67] Nevertheless, its detection methods need to rely on cutting-edge technologies, such as second-generation sequencing, and qRT- PCR. At the same time, its detection has high requirements for testers, which limits its wide application in clinic.

Conclusions

This paper reviews the roles of CTCs in diagnosis, monitoring and prognosis of ESCC, and provides a powerful basis for early diagnosis, recurrence monitoring and prognosis evaluation of ESCC as well as the possibility for individualized treatment of ESCC. The content of CTCs in ESCC is higher than that in normal controls, and is closely related to the depth of tumor invasion, lymph node metastasis and disease stage. A large number of experiments show that CTCs are associated with poor PFS and OS especially when CTC>2. Early monitoring of CTCs may reduce the risks of recurrence and metastasis, and thus improve the prognosis of ESCC patients. Therefore, real-time non-invasive CTC monitoring is of great significance for the diagnosis and treatment of ESCC. The changes of CTC values before and after treatment can guide clinical practice and further help to realize individualized treatment. However, due to the limitations of CTC detection technology and the interference of EMT, which limit the wide use of CTCs in clinic.But for the unable to second-generation sequencing or qRT-PCR institutions, CTC detection is relatively simple.Although the roles of CTC are studied in various tumors and are guiding, further large-scale research on CTCs in ESCC is still needed to realize standardized management.

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Disclosure

The authors report no conflicts of interest in this work.

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