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Circulating Tumor Cells Correlate With Prognosis in Head and Neck Squamous Cell Carcinoma

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Abstract

Background: To investigate the relationship of circulating tumor cells (CTCs) and the clinical characteristic parameters and prognosis in patients with head and neck squamous cell carcinoma (HNSCC). **Methods:** The retrospective clinical study included 95 patients with HNSCC who after surgery in Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine between December 2015 and December 2016. All patients were followed up for survival until the end of June 2019. The CTCs detection was performed by negative enrichment (NE) immunofluorescence-in situ hybridization (im-FISH) of chromosome 8. **Results:** Patients with higher CTCs counts are associated with a worse prognosis with an area under the receiver operator characteristic (ROC) curve of 0.756 [95% confidence interval (CI) 0.640-0.872, $p = 0.001$]. The CTCs-positive rate of HNSCC patients was 58.9% (56/95) by using the cut-point of 3. Both the chi-square test and binary logistic regression analysis showed that the N stage and clinical stage were significantly associated with CTCs-positive in patients with HNSCC ($p < 0.05$). Further Non-parametric test analysis indicated that more CTCs counts were detected in late N and clinical stages patients ($p < 0.001$). The Kaplan-Meier survival analysis indicated that CTCs-positive were correlated with shorter progression-free survival (PFS) ($p < 0.001$) and overall survival (OS) ($p = 0.001$). Further, the CTCs-positive was an independent prognostic factor for PFS and OS according to the Cox multivariate regression analysis ($p < 0.05$). **Conclusion:** More CTCs were associated with N stage, clinical stage, poor prognosis in patients with HNSCC, which might be used as a prognostic biomarker.

Keywords

circulating tumor cells, head and neck squamous cell carcinoma, prognosis, survival, negative enrichment-fluorescence in situ hybridization

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Background

Head and neck neoplasms are the sixth most common malignancy worldwide, of which about 90% is head and neck squamous cell carcinoma (HNSCC).[1] The prognosis of HNSCC has been improved with the advancement of surgery, adjuvant radiotherapy, and concurrent chemoradiotherapy (CRT), but the 5-year overall survival (OS) rate is still less than 50%.[2] Local recurrence and metastasis, remain the major causes of HNSCC-progression, which is mostly through lymph node metastasis.[3] Therefore, the detection of metastasis at early stage is crucial for clinical management. Pathological diagnosis, performed as a gold standard for lymph node metastasis, is traumatic and might cause tumor spread during operation.[4] More importantly, the monitoring of lymph node alone does not indicate that the metastases of the tumor could be found, as some HNSCC patients with early N stage might also have distant metastasis through circulation. Hence, it is of great

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importance to find an effective biomarker to monitor the metastases and predict the prognosis of HNSCC.

Circulating tumor cells (CTCs) are rare which detaching themselves from solid tumors and intravasating into the bloodstream, from where they are carried to other organs and are responsible for the generation of distant metastases. Thus, CTCs were considered as the media of metastasis.[5] CTCs was proposed by Ashworth since 1869, subsequently, it was confirmed that CTCs could provide real-time information for monitoring the development of malignant epithelial tumors, such as breast cancer, colorectal cancer, and prostate cancer.[6-8] Over the past 30 years, many independent studies have provided evidence of hematogenous tumor cell dissemination in HNSCC and the potential role of CTCs in disease progression.[9] But the correlation between CTCs and the clinicopathological characteristics, the prognosis of the HNSCC patients has not yet been verified clearly. We hypothesize that the detection of CTCs in HNSCC patients might help to predict recurrence and/or metastasis and detection of metastasis timely could better improve the overall management of HNSCC patients.

The negative enrichment (NE) combined with immunofluorescence and in situ hybridization (NE-imFISH) is an epithelial cell adhesion molecule (EpCAM)-independent technique, which has been reported effective for detecting CTCs in some solid tumors.[10,11] This study aimed to analyze CTCs in HNSCC patients by using the NE-imFISH technology, and further to explore the relationships between CTCs and clinicopathological characteristics, prognosis in HNSCC patients.

Subjects and Methods

Patients

This clinical retrospective study was conducted in the Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University of medicine, which is one of the largest HNSCC outpatients in China. Firstly, a total of 100 patients with HNSCC who underwent operation or biopsy were recruited from December 2015 and December 2016, of which 5 subjects who were lost to follow-up were excluded. There were 58 males (68.9%) and 37 females (31.3%), the median age was 60 years old with a range from 24 to 86. The primary sites of HNSCC in the sample mainly included oral cavity, oropharynx, hypopharynx, larynx and a few cases of lip cancer, ethmoid sinus tumor, and maxillary sinus tumor etc, excluding nasopharyngeal carcinoma. The clinical stages of HNSCC patients were based on the criteria for HNSCC stage formulated in the eighth edition of American Joint Committee on Cancer (AJCC) 2017, including 4 cases (4.2%) with stage I, 12 cases (12.6%) with stage II, 14 cases (14.7%) with stage III, and 65cases (68.4%) with stage IV. The inclusion criteria were as follows. (1) Patients diagnosed as HNSCC by newly clinical and histopathological examinations. (2) Patients diagnosed without primary malignant tumors at other locations. (3) Patients underwent surgery without neoadjuvant chemotherapy or radiotherapy. (4) Patients had no concurrent serious diseases that might affect the prognosis. Patient characteristics are summarized in Table 1. The study was approved by the Ethics Committee of the Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine (approval no. SH9H-2019-T270-2) and was performed according to the Declaration of Helsinki principles. Due to the retrospective nature of the study, patient consent for inclusion was waived.

The endpoints were OS and PFS. The OS was measured from the time elapsed from CTCs detection to death, and the PFS referred to the date of the time elapsed from CTCs detection to disease progression or death. Tumor response was evaluated by computed tomography (CT) scanning or magnetic resonance imaging (MRI) according to the response evaluation criteria in solid tumors (RECIST 1.1). The patients were followed up to June 2019 or their death through telephone, outpatient, or inpatient records, every 3 months for the first years and every 6 months thereafter.

Enrichment and Isolation of Circulating Tumor Cells

Enrichment and isolation of CTCs were performed using CD45-antibody which combined the fluorescence in situ hybridization with chromosome 8 centromere probes (Cyttel Biosciences INC, Jiangsu, China). Blood samples were collected at 1 month post-operation or biopsy but before the following therapy. In brief, blood was collected in vacuum blood collection tubes from the median cubital vein of the patients in the morning. 7.5 ml of venous blood was collected in the ACD anticoagulant tube and kept at room temperature no more than 2 hours. All specimens were treated within 48 hours. The samples were washed with CS1 buffer (Cyttel) and centrifuged at 700 g for 5 min at room temperature, and red blood cells were removed successively through lysates. The supernatant was discarded and erythrocytes were lysed by adding CS2 buffer (Cyttel), and the mixture was sequentially spun down at 700 g for 5 min at room temperature. Samples were transferred to CS3 (Cyttel) and separated by gradient centrifugation at 300 g for 5 minutes. According to a previously published protocol,[12] the cell suspension was added to CS1 buffer (Cyttel), and the immunomagnetic particles conjugated anti-CD45 antibody (Cyttel) were sequentially added and mixed well. And then, the specimen was placed on a magnetic stand for 10 minutes. The supernatant was extracted from the magnetic field and centrifuged at 900 g for 5 minutes, and rinsed with CS1 buffer (Cyttel), then incubated with anti-CD45 conjugated to Alexa Fluor 594 (red, Cyttel) for 1 hour in the dark. The cells were fixed by cell fixatives-1, and Centromere Probe (CEP) 8 (red, Cyttel) was used for fluorescence in situ hybridization. At last, the specimens were rinsed with Phosphate Buffered Saline (PBS) covered with mounting medium containing 4′,6-diamidi-no-2-phenylindole (DAPI), and observed by fluorescence microscope.

The CTCs-positive is defined as hyperdiploid $CEP8^{+}/DAPI^{+}/CD45^{-}$. The CEP8-positive is defined as CEP8 signal points are equal to two or more. Additionally, if the distance between 2 CEP8 signal points is less than 1 signal diameter, it

is considered as 1 signal according to the manufacturer's instructions.

Statistical Analysis

Data were analyzed by SPSS 22.0 (IBM, Armonk, NY, USA) statistical software and graphs were plotted by GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Pearson chi-square test or Fisher exact method was used to test the differences in CTC-positive and CTC-negative groups, while the associations between CTCs and clinicopathological variables were calculated by Mann-Whitney U test, and the result was calculated by 2 sided test. Multivariate analysis of the relation between CTC-positive and CTC-negative groups was calculated by Binary logistic regression. Survival curves were obtained by Kaplan-Meier survival analysis and compared by log-rank test. Both univariate and multivariate analyses were performed to assess the relationship between survival and several variables simultaneously using Cox proportional hazards mode. $P < 0.05$ was considered to be statistically significant.

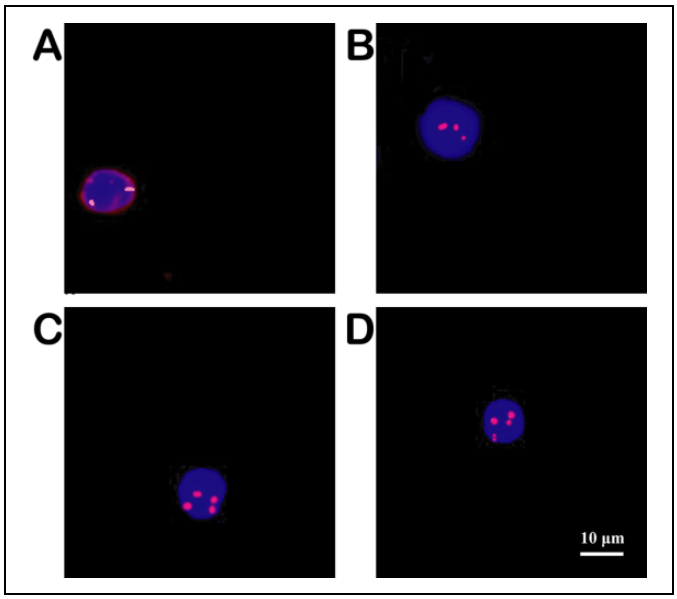

Figure 1. CTCs morphology stained by immunofluorescence (im-FISH×400). Identification of CTCs in patients with HNSCC by NE-imFISH technology. CTCs were defined as hyperdiploid $CEP8^{+}$/$DAPI^{+}$/$CD45^{-}$; (A) White blood cell; (B) Triploid CTC; (C) Tetraploid CTC; (D) Polyploid CTC.

Results

Efficiency of CTCs Detection in HNSCC Patients

Of 95 subjects with HNSCC, 77 were detected with CTCs, and the median number of CTCs was 7.5, range from 0 to 13 CTCs/7.5 ml. The CTCs detection rate was 81.1%. Since there is no international standard for CTCs-positive at present, cut-point of ROC for PFS and OS which was used to assess prognosis was regarded as the CTC-positive in HNSCC. And the area under ROC curve (AUC) for PFS and OS were 0.756 and 0.640, respectively (Figure 2). Given that the larger AUC means stronger predictive power, we combined Yourdon's index and the AUC for PFS. The CTCs-positive rate of HNSCC was 58.9% (56/95) in this study, when introduced an optimal cut-point of 3 CTCs/7.5 ml (AUC = 0.756, 95% CI, 0.640-0.872, p = 0.001). The results indicated that the CTCs detection rate and CTCs-positive rate were relatively high in patients with HNSCC using NE-imFISH method.

The Association Between CTCs and Clinicopathologic Characteristics in HNSCC Patients

The relationships between CTCs and clinicopathologic characteristics are shown in Table 2. Univariate analysis showed that CTCs-positive were significantly correlated with tumor differentiation ($p = 0.004$), N ($p < 0.001$) and clinical stage ($p <$

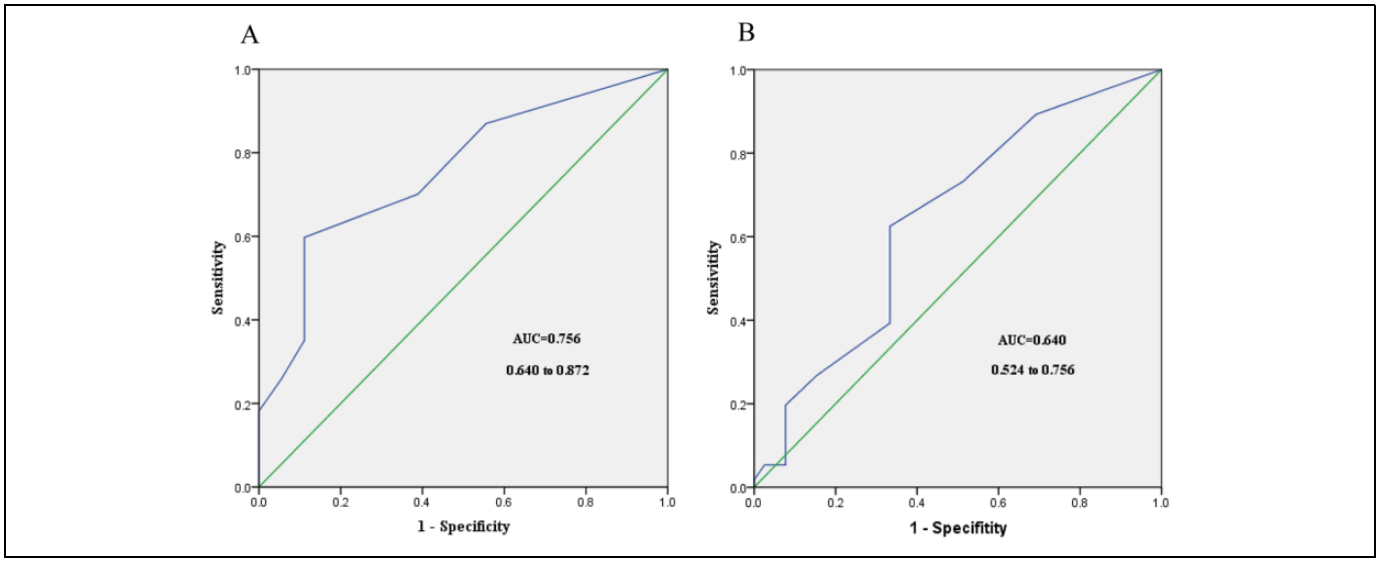

Figure 2. ROC curves for CTCs count to assess prognosis. (A) AUC for PFS; (B) AUC for OS. AUC indicates area under the curve.

Table 1. Basic Characteristics of Patients With HNSCC.

Characteristics	n	Percentage(%)
Age(years)		
≤60	50	52.6
>60	45	47.4
Gender		
Male	58	61.1
Female	37	38.9
Tumor site		
Oropharynx	10	10.5
Oral cavity	37	38.9
Hypopharynx	16	16.9
Larynx	20	21.1
Others	12	12.6
Tumor grade		
poor	35	36.8
well to moderate	60	63.2
HPV status		
Negative	49	51.6
Positive	32	33.7
Unknown	14	14.7
T stage		
T_1+T_2	86	90.5
T_3+T_4	9	9.5
N stage		
N_0	19	20.0
$N_1+N_2+N_3$	76	80.0
M stage		
M_0	69	72.6
M_1	26	27.4
Clinical stage		
I+II	16	16.8
III+IV	79	83.2
Treatment		
Chemoradiotherapy	56	58.9
Others	39	41.1

0.001) of patients with HNSCC, whereas independent of age, gender, tumor site, HPV status, T and M stage ($p > 0.05$). Multivariate binary logistic regression showed that only N ($p = 0.001$; OR, 0.008; 95%CI: 0.020-0.385; Table 2) and clinical stage ($p = 0.002$; OR, 0.033; 95%CI: 0.004-0.299; Table 2) were positively correlated with CTCs-positive, no significant correlation was found in age, gender, tumor differentiation, HPV status, T and M stages ($p > 0.05$). Besides, further analysis of the numbers of CTCs in samples with different N or clinical stage showed that the median numbers of CTCs detected were 0.73 ± 1.45 and 3.82 ± 2.83 (CTCs/7.5 ml) in N_0 and $N_1+N_2+N_3$, respectively. The median number of CTCs in stage I+II and III+IV HNSCC patients were 0.38 ± 0.72 and 3.77 ± 2.82 (CTCs/7.5 ml), respectively. The above results revealed that more CTCs numbers were detected in the late N stage or clinical stage ($p < 0.001$, Figure 3).

Correlation of CTCs and Prognosis in HNSCC Patients

The median PFS and OS were 8 months (range from 1 to 36 months) and 18 months (range from 1 to 36 months), respectively. In univariate survival analysis, tumor differentiation, N stage, M stage, clinical stage, and CTCs-positive were negatively correlated with the PFS and OS in patients with HNSCC ($p < 0.05$). The PFS rate of CTCs-negative and CTCs-positive were 38.5% and 5.40%, respectively. Besides, the OS rate of CTCs-Negative and -positive group were 64.7% and 25.0%, respectively (Figure 4, Table 3). Additionally, we further stratified the patient by whether or not the tumor had metastasized. The Kaplan–Meier survival curves of 69 non-metastatic patients showed that HNSCC patients with CTC-positive still had a significantly unfavorable prognosis (Figure 5). The results mentioned above indicated that HNSCC patients with CTCs-positive might have poor PFS and OS than patients with CTCs-negative. Eventually, multivariate Cox regression analyses showed that only the CTCs-positive and M stage of HNSCC were the independent prognostic factors of the unfavorable PFS and OS ($p < 0.05$, Table 4).

Discussion

HNSCC is a complex disease with tumor heterogeneity and genomic complexity. There are nearly half a million newly diagnosed patients with HNSCC every year. Despite the timely updated medical concept and therapeutic modalities, there are still over thirty million deaths every years.[13] One reason for the high mortality rate of HNSCC is that the patient was diagnosed late, and 40% of the patients were initially diagnosed as stage III-IV while 10% of which had been diagnosed with distant organ metastases.[14] Therefore, to facilitate individual therapeutic interventions, early detection, and diagnosis of HNSCC are becoming necessary. CTCs detection has been widely accepted as a "liquid biopsy" with the advantage of non-invasivity, real-time and rapid, which plays an important role in the auxiliary diagnosis, treatment assessment, and prognosis judgment of some epithelial tumors.[15] Although it has undisputable implications, there is still a lack of evidence about the analytical and clinical validation of CTCs in patients with HNSCC. Thus, this study regarding the relationship between CTCs and the prognostics of HNSCC patients will offer help for individualized treatment and have prognosis value among HNSCC patients.

CTCs usually fall into the blood from the primary sites by means of epithelial-mesenchymal transition (EMT).[16] Through apoptosis or being swallowed up by immune cells, only a handful of CTCs survive and metastases, so CTCs cannot be detected easily.[17] At present, there are many kinds of methods to detect the CTCs, including polymerase chain reaction (PCR) techniques, flow cytometry, microfluidic chips, immunomagnetic enrichment and so on.[18] Only the CellSearch system (Menarini Silicon Biosystems, Bologna, Italy) based on EpCAM is approved by the US Food and Drug Administration, which is widely used in breast cancer, prostate cancer and colorectal cancer.[19] It is a clinical tool to stratify patients into different treatment groups based on risk of metastasis, monitor response to therapy and outline prognosis. Only by enumerating the CTCs after isolation and identification accurately can the patient prognosis be effectively assessed.[20] There are

Table 2. The Relationships Between CTCs-Positive and Clinicopathologic Characteristics in HNSCC Patients.[a]

Clinicopathological characteristics	n (%)	CTCs (n)		Univariate analysis		Multivariate analysis	
		CTCs (+)	CTC (−)	χ^2	p	OR[95%CI]	p
Age				2.105	0.147	N	N
≤60	50(52.6)	26	24				
>60	45(47.4)	30	15				
Gender				0.259	0.611	N	N
Male	58(68.9)	33	25				
Female	37(31.1)	23	14				
Tumor site				4.144	0.387	N	N
Oropharynx	20(21.1)	13	7				
Oral cavity	37(38.9)	24	13				
Hypopharynx	16(16.9)	7	9				
Larynx	12(12.6)	8	4				
Others	10(10.5)	4	6				
Tumor differentiation				8.211	0.004	0.757 [0.257-2.233]	0.614
well	35(36.8)	14	21				
moderate to poor	60(63.2)	42	18				
HPV status				2.065	0.356	N	N
Negative	49(51.6)	30	19				
Positive	32(33.7)	16	16				
Unknown	14(14.7)	10	4				
T stage				0.019	0.890	N	N
T_1+T_2	86(90.5)	50	36				
T_3+T_4	9(9.5)	6	3				
N stage				18.280	<0.001	0.088 [0.020-0.385]	0.001
N_0	19(20.0)	3	16				
$N_1+N_2+N_3$	76(80.0)	53	23				
M stage				0.613	0.434	N	N
M_0	69(72.6)	39	30				
M_1	26(26.4)	17	9				
Clinical stage				22.079	<0.001	0.033 [0.004-0.299]	0.002
I+II	16(16.8)	5	11				
III+IV	79(83.2)	65	6				

[a]Univariate analysis: Pearson chi-square test or Fisher exact probability method; multivariate analysis: logistic regression analysis. N: Not done.

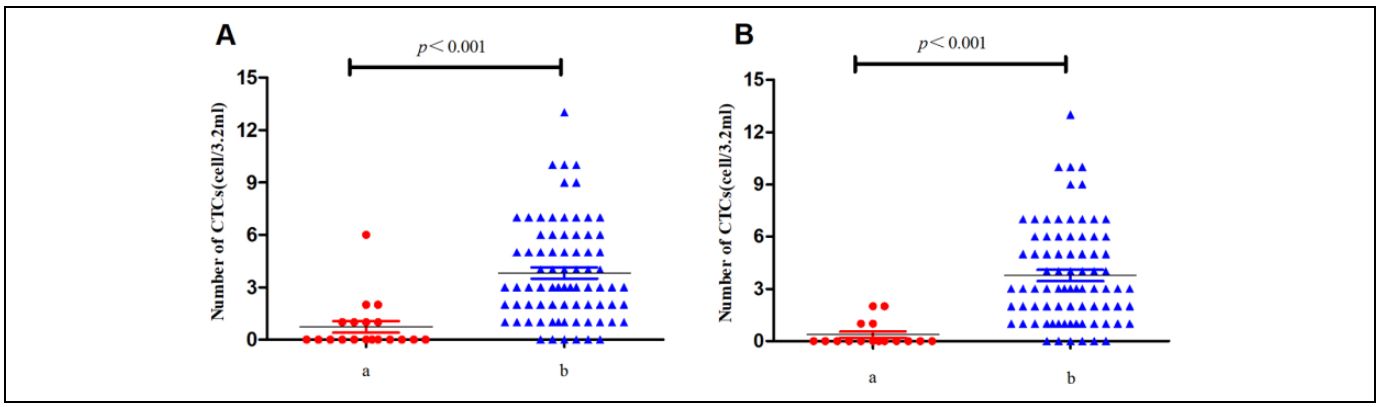

Figure 3. CTCs counts in patients with different N stage (A), patients with N_0 stage (a) and patients with $N_1 \sim N_3$ stages (b). CTCs counts in patients with different clinical stages (B), patients with I ~ II stages (a) and patients with III ~ IV stages (b).

certain limitations that EpCAM would be down-regulated or secreted during the EMT course with respect to CTCs identification.[21] Due to the absence of EpCAM in the EMT process, CTCs in the EMT process cannot be detected so that false negative occurs. Nichols et al. successfully detected CTCs in HNSCC patients by using CellSearch system, but the positive rate was low (6/15).[22] The results are given above also explained the effect of EpCAM down-regulation on CTC detections, and showed that CellSearch system might not be suitable for HNSCC.

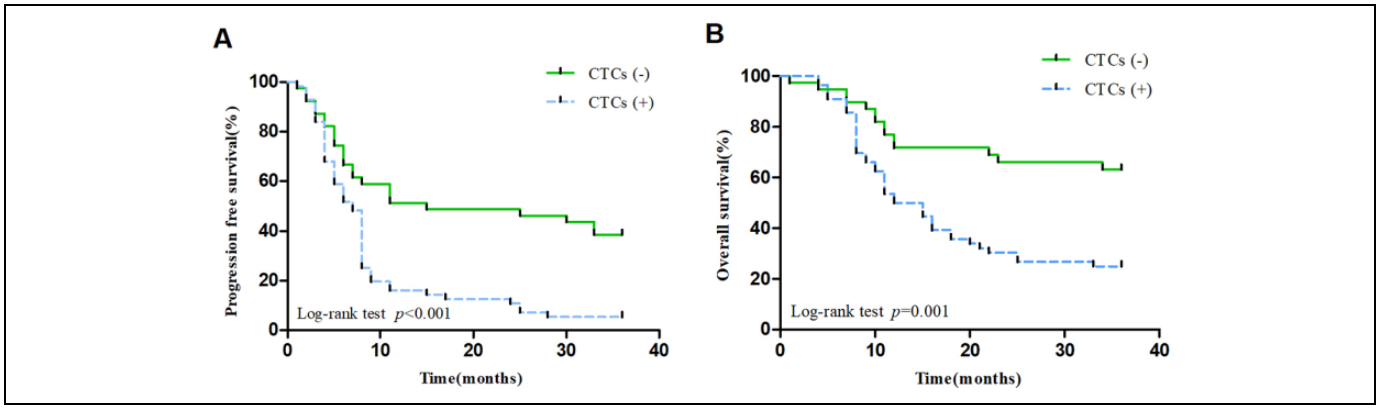

Figure 4. Kaplan-Meier curves of PFS (A) and OS (B) in HNSCC patients with CTCs positive and negative.

Table 3. The Univariate Survival Analysis in HNSCC Patients.

Characteristics	PFS (%)	Log rank value	*p*	OS (%)	Log rank value	*p*
Age		1.636	0.201		2.107	0.147
≤60	24.0			50.0		
>60	13.3			31.1		
Gender		0.014	0.906		0.066	0.798
Male	22.4			39.7		
Female	13.5			43.2		
Tumor site		5.854	0.210		5.380	0.250
Oropharynx	20.0			40.0		
Oral cavity	10.8			40.5		
Hypopharynx	37.5			50.0		
Larynx	8.3			16.7		
Others	30.0			60.0		
Tumor differentiation		8.849	0.003		18.344	<0.001
well	31.4			71.4		
moderate to poor	11.7			23.3		
HPV status		2.292	0.318		2.784	0.249
Negative	25.0			53.1		
Positive	14.3			32.7		
Unknown	16.3			42.9		
T stage		0.680	0.410		0.699	0.403
T_1+T_2	18.6			39.5		
T_3+T_4	22.2			55.6		
N stage		10.441	0.001		12.261	<0.001
N_0	47.4			78.9		
$N_1+N_2+N_3$	11.8			31.6		
M stage		23.776	<0.001		23.828	<0.001
M_0	23.2			52.2		
M_1	7.7			11.5		
Clinical stage		12.089	0.001		13.389	<0.001
I+II	56.3			87.5		
III+IV	11.4			31.6		
CTCs		15.062	<0.001		12.203	0.001
Negative	38.5			64.7		
Positive	5.40			25.0		
Treatment		1.748	0.186		0.722	0.395
Chemoradiotherapy	12.5			35.7		
Others	23.1			46.2		

Abbreviation: PFS, Progression free survival;OS: Overall survival.

In this study, we applied a new capture technique (NE–imFISH) to identify the cells with hyperdiploid $CEP8^+/DAPI^+/CD45^-$ as positive CTCs in HNSCC. This method regardless of the expression of epithelial markers such as EpCAM and cytokeratin (CK), which is applicable to most solid tumors, and significantly improve the detection rate of

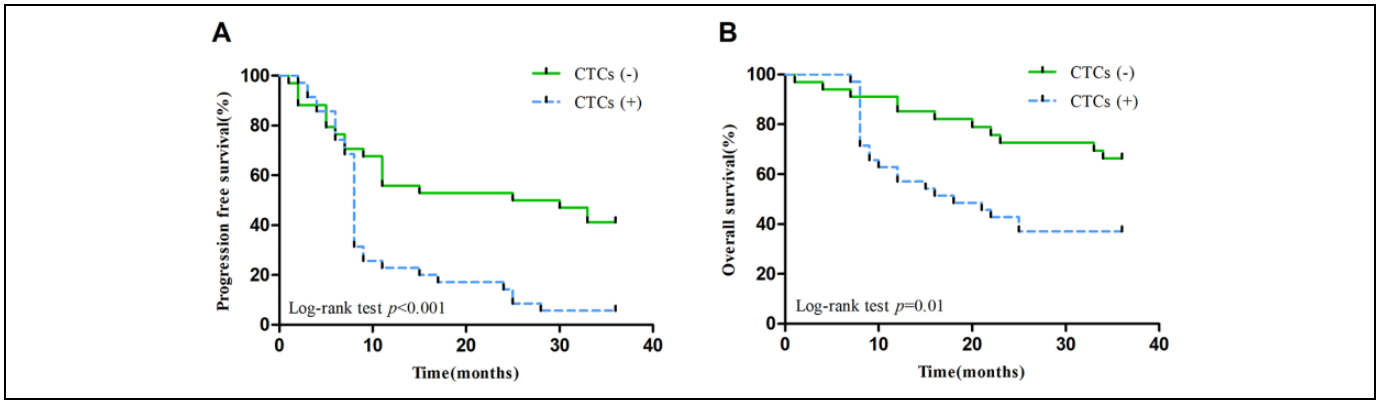

Figure 5. Kaplan-Meier curves of PFS (A) and OS (B) in non-metastatic HNSCC patients with CTCs positive and negative.

Table 4. Cox Proportional Hazards Mode in HNSCC Patients.

	PFS			OS		
Characteristics	HR	95%CI	*p*	HR	95%CI	*p*
M stage						
M_0		reference			reference	
M_1	3.603	1.936-6.705	<0.001	2.539	1.376-4.684	0.003
CTCs						
Negative		reference			reference	
Positive	2.597	1.474-4.574	0.001	2.036	1.072-3.867	0.030

Abbreviation: CI, Confidence interval; PFS, Progression free survival;OS: Overall survival; HR, Hazard ratio.

CTCs. In recent research on HNSCC, the positive detection rate of CTCs fluctuated greatly due to the difference between the determination criteria and the detection method, which was generally between 6.0% and 89.0%.[9] Kulasinghe et al used a novel straight microfluidic chip, based on FISH, to capture CTCs.[23] CTC clusters and circulating tumor microemboli (CTM) were identified successfully, which were not observed by NE-imFISH in our study. While the positive detection rate of CTCs was only 47.6% (10/21), which owing to the limitation of this technology that small CTCs which sizes are similar to white blood cells are unlikely to be captured.[24] As mentioned above, the CTCs detection rate and CTCs-positive rate were 81.1% and 58.9% respectively in HNSCC patients, which demonstrated that NE–imFISH has higher sensitivity and specificity in CTCs detection compared with conventional technologies. Meanwhile, NE-imFISH could incorporate multiple chromosome probes like CEP7 and CEP17 to cater to CTCs heterogeneity, so this strategy could be extended to other cancer types. NE-imFISH also successively demonstrated its advantages in improving CTCs detection rate in other solid tumors.[25] In addition, compared with other methods, it has the advantages of shorter detection time and lower volume of peripheral blood. NE-imFISH wouldn't change the specific antigen expression on the surface of CTCs, so that CTCs can maintain high activity, which is beneficial for the research on morphology and function of CTCs.[9]

In patients with breast, prostate, or colorectal cancer, the detection of CTCs or baseline CTCs levels were assessed as biomarkers of cancer progression. Until now, numerous studies on the relationships between CTCs and clinical characteristics of patients with HNSCC were controversial. Buglione et al found that, among 73 HNSCC patients, CTCs-positive rate of stage IV patients was higher than that of stage I-III patients (18% vs 6%), but the difference was not significant.[26] Grobe et al showed that the positive rate of CTCs was only related to T and M stage,[27] while Hsieh et al concluded that CTCs quantity was only related to N stage, rather than T or M stage.[28] On the contrary, Wu et al concluded that there was no significant correlation between CTCs and gender, age, T stage, N, and clinical stage in HNSCC patients through a meta-analysis of 23 studies on CTCs.[29] The difference between the above studies might own to a relatively small number of patients, which highlighted the characteristics of tumor heterogeneity in different populations. And it might also be attributed to the lack of standardized CTCs detection methods and differences in patients statistical grouping. Compared with previous studies, we have a larger number of enrolled samples as well as a more sensitive detection method. By analyzing the relationship between clinicopathologic characteristics and CTCs in HNSCC patients, we found that CTCs counts were correlated with Lymph node metastasis and disease progression, which might be as a potential intervention technique of personalized medicine and might also improve the prognosis of the HNSCC patients.

In addition, the prediction of the prognosis among HNSCC patients is also particularly important. Recently, a number of studies have also investigated the correlation between CTCs and the prognosis of HNSCC patients. Buglione et al found that the positive rate of CTCs was not correlated with the recurrence, treatment, and survival of HNSCC.[26] Nevertheless, a series of articles on HNSCC have shown that CTCs are associated with the prognosis of HNSCC patients.[30,31] They thought that the detection of CTCs accounts for faster progression as well as worse prognosis, and the number of CTCs could change with treatment. In the latest study, Morgan et al used surface-enhanced Raman scattering (SERS) nanotechnology

identified amounts of CTCs in HNSCC.[32] They demonstrated that the number of CTCs was correlated with the disease progression of HNSCC patients, and the level of CTCs was a strong indicator of poor prognosis. In this study, we found that PFS and OS of HNSCC patients with CTCs-positive were shorter than those of CTCs-negative patients by survival analysis, which is consistent with most of the previous studies. On the whole, the results potentially indicate that CTCs means the metastasis and development of HNSCC, which is in accordance with that CTCs means the spread of tumors.[33] Moreover, CTCs-positive was an independent prognostic factor for OS and PFS, which means that CTCs detection has potential value in HNSCC.

This study still had some limitations. Our study is a retrospective study and the total number of cases was still small. CTCs were collected at a single time-point without comparing between pre-treatment and post-treatment. Additionally, almost all of patients were diagnosed with advanced disease, which is associated with poor prognosis. Meanwhile, the median follow-up time of HNSCC patients was only 32 months, and many patients were still in the process of follow-up. Further studies are needed to correct these deviations by expanding the sample size and follow-up time.

Conclusion

This study showed that CTCs-positive is related to advanced N and clinical stage in HNSCC patients. Further, CTCs-positive could predict shorter PFS and OS. Thus, the detection of CTCs in HNSCC patients might be used as a predictive biomarker of the tumor spreading in HNSCC patients.

Authors' Note

Shichao Zhou and Lili Wang contributed to the work equally. J Wang and Y Zhang designed the study. H Yuan, L Wang and F Liu collected the samples. J Wang, W Zhang and H Yuan followed up the OS and PFS of the patients. B Jiang, S Zhou, H Yuan and L Wang analyzed the data. S Zhou wrote the manuscript with assistance from J Wang and H Yuan. The final approval were from all authors.

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Declaration of Conflicting Interests

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