Application of CellSearch technique in detection of peripheral blood circulating tumour cell count in patients with head and neck cancer and its association with prognosis

HAIYANG LI[1], PINGDONG LI[2,3], FENG LI[1] and TAO WANG[1]

[1]Department of Otolaryngology, Beijing Daxing District People's Hospital, Beijing 102600, P.R. China; [2]Department of Otolaryngology, Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, P.R. China; [3]Key Laboratory of Otorhinolaryngology, Head and Neck Surgery, Ministry of Education, Beijing Institute of Otorhinolaryngology, Beijing 100730, P.R. China

Received April 23, 2024; Accepted September 12, 2024

DOI: 10.3892/ol.2024.14846

Abstract. The aim of the present study was to employ CELLSEARCH® technology for the detection of circulating tumor cells (CTCs) in the peripheral blood of head and neck cancer (HNC) patients, and to assess the association between CTC count and patient prognosis. In this retrospective study, a cohort of 56 patients diagnosed with HNC and receiving treatment at the Department of Otolaryngology, Head and Neck Surgery (Beijing Tongren Hospital) between December 2013 and June 2018 were selected. Utilizing CELLSEARCH® technology, the presence of CTCs were detected in samples of peripheral blood from patients with head and neck cancer (HNC) patients, and CTC counts were documented. CTC positivity was defined as CTCs ≥1/7.5 ml of peripheral blood. Comprehensive data encompassing general demographic profiles, pathological classifications, tumor node metastasis (TNM) staging, tumor histology and treatment modalities were gathered for each participant. The study employed the Kaplan-Meier method to scrutinize and compare survival rates between CTC-positive and CTC-negative cohorts, while both univariate and multivariate Cox regression analyses were conducted to discern the factors impacting the overall survival (OS) of individuals diagnosed with HNC. Out of the 56 patients examined, 14 individuals exhibited detectable levels of CTCs, resulting in a positivity rate of 25%. The analysis revealed a significant association between the levels of CTCs in patients with HNC and the utilization of non-surgical treatment (P<0.05), while no substantial associations were observed concerning sex, age, smoking habits, alcohol consumption, pathological classifications, TNM staging, tumor attributes and surgical interventions (all P>0.05). Survival analysis revealed a reduction in the OS among patients with HNC harboring CTC positivity in contrast to their CTC-negative counterparts. The comprehensive multivariate Cox regression analysis underscored the independent prognostic impact of CTC presence (HR=1.274; 95% CI, 1.119-1.451; P<0.001) and the implementation of non-surgical treatment (HR=0.268; 95% CI, 0.119-0.607; P=0.002) on the prognosis of individuals grappling with HNC. In conclusion, the levels of CTCs were an independent factor affecting outcomes in patients with HNC, with CTC-positive patients showing significantly shorter survival compared with CTC-negative cases.

Correspondence to: Professor Haiyang Li, Department of Otolaryngology, Beijing Daxing District People's Hospital, 26 Huangcun West Street, Beijing 102600, P.R. China
E-mail: dxlihaiyang@163.com

Abbreviations: CD45, cluster of differentiation 45; CK, cytokeratin; CTCs, circulating tumor cells; DFS, disease-free survival; EpCAM, epithelial cell adhesion molecule; HNC, head and neck cancer; HNSCC, head and neck squamous cell carcinoma; OS, overall survival; SCC, squamous cell carcinoma

Key words: CellSearch technique, HNC, CTCs, prognosis

Introduction

Head and neck cancer (HNC), the seventh most common type of cancer worldwide, consists of numerous tumors affecting the upper respiratory tract (1). Although there are a number of different histological types, squamous cell carcinoma (SCC) consists of ~95% of cases, covering tumors of the lips, mouth, nasal cavity, sinuses, pharynx and larynx (2). The major risk factors include smoking, alcoholism and carcinogenic virus, as well as human papilloma and Epstein-Barr viruses (3,4). HNC, which is often diagnosed in patients who use tobacco and alcohol heavily, presents a slowly declining incidence globally, in part due to a decrease in tobacco use (5).

Over the past few decades, a comprehensive treatment mode that integrates surgery, radiotherapy and chemotherapy has been formed and developed, which has significantly improved the local control rate of tumors but not the overall survival (OS), with the 5-year OS rate remaining at only 40-50% (6-8). Treatment of head and neck malignancies remains challenging and requires a multidisciplinary approach. In this scenario, a multi-specialty team evaluation is critical in determining the treatment options for HNCs (9). Due to the special anatomical structure of HNC, early diagnosis and early detection of

tumor recurrence are still difficult, various forms of endoscopy and tumor tissue biopsy are the current gold standard for diagnosis (10). However, this invasive test has a number of drawbacks, including the inability of patients to tolerate multiple biopsies, the high cost, the inability to make a definitive diagnosis in difficult-to-sample areas and the inability to consider the heterogeneity of the tumor when a single sample is used to diagnose the disease (11). Therefore, there is an urgent need for convenient and accurate tests that can identify patients at high risk of metastasis before the occurrence of metastatic lesions, thereby improving the ability to individualize treatment for patients.

Circulating tumor cells (CTCs) are shed from primary tumors into the bloodstream, where they may seed metastases in distant organs (12,13). Detecting CTCs serves as a valuable biomarker for cancer progression and offers a target for early detection, prognosis and monitoring treatment efficacy (14). Despite their infrequency, with counts typically below 10 CTCs per 7.5 ml of blood in patients with solid tumors, advancements in microfluidics and nanotechnology are enhancing detection sensitivity and specificity (15-18). The CELLSEARCH® (Menarini Silicon Biosystems, Inc.) system, employing immunomagnetic enrichment and fluorescent labeling, is a prevalent method for CTC identification. CTC enumeration is a prognostic indicator in various types of cancer, including metastatic breast, prostate and colorectal, and fluctuations in CTC levels can signal treatment responses and survival outcomes (19).

However, several challenges impede the clinical application of CTCs. Their rarity complicates detection and analysis. Additionally, the heterogeneity of CTCs necessitates standardized protocols for isolation and analysis. The clinical implications of single CTCs versus clusters also require further clarification (20).

Looking ahead, the integration of CTC analysis with other components of liquid biopsies, such as cell-free DNA, will provide a more comprehensive assessment. The development of more sensitive and specific technologies for CTC detection and analysis is a priority. In essence, CTCs are a promising frontier in oncology, with the potential to significantly enhance cancer diagnosis, treatment and monitoring. The ongoing research and development in this area are expected to yield discoveries that will improve patient care and outcomes.

The present retrospective study aimed to investigate the relationship between CTC count in the peripheral blood of patients with HNC and patient outcomes, with the goal of offering insights into the potential use of CTCs as a prognostic marker for this patient population.

Materials and methods

Study subjects. The clinical data of 56 cases of patients pathologically diagnosed with HNC that were admitted between December 2013 to June 2018 to the Department of Otolaryngology, Head and Neck Surgery (Beijing Tongren Hospital, Capital Medical University, Beijing, China) were retrospectively reviewed (Table I). The diagnosis was confirmed by a pathologist, who was independent from the study. The inclusion criteria were as follows: i) All patients were pathologically diagnosed with HNC; ii) no distant organ metastasis as confirmed by routine imaging examination; and iii) complete clinical data and follow-up information. The exclusion criteria were as follows: i) Malignancies in other organs; ii) hematological disorders and/or immune system diseases; iii) perioperative death or death caused by non-tumor reasons; iv) patients requiring long-term oral anticoagulation drugs; and v) incomplete clinical data and/or follow-up information. The study was approved by the hospital ethics committee of Beijing Tongren Hospital, Capital Medical University.

CTC detection. CTC detection was performed at the time of sample collection as part of routine clinical testing. The CellSearch technique (21), utilized by Nuohai Life Science (Shanghai) Co. Ltd, is a non-invasive diagnostic method for detecting and quantifying CTCs in the peripheral blood of cancer patients. The system comprises the Celltracks Autoprep, Celltracks Analyzer II and CellSave Tube Preservative tubes and kits, which include epithelial cell adhesion molecule (EpCAM) antibody-coated immunomagnetic beads, fluorescent antibodies for cytokeratin (CK) and CD45), cell fixatives and buffers. i) Specimen Collection: Blood samples were collected from patients on an empty stomach before surgery or treatment, with the first 1 ml discarded to avoid contamination. A 10 ml sample was drawn into a CellSave tube, mixed, stored at room temperature and tested within 72 h. ii) Procedure: 7.5 ml of peripheral blood was transferred into a Cell Search conical tube, followed by the addition of 6.5 ml of diluent. After centrifugation at 800 g for 10 min, the sample was processed in the CellTracks Autoprep System. The system automatically enriched CTCs using antibodies and DAPI for co-incubation, following the instrument's protocol. iii) Detection process: Blood samples were collected (7.5 ml, and CTCs were enriched through immunomagnetic separation based on EpCAM expression, a molecule selectively present on epithelial cells including CTCs. iv) Identification: Post-enrichment, CTCs were identified with fluorescently labeled CK antibodies and specific epithelial cell markers. DAPI staining visualized the nucleus, confirming intact cells. v) Exclusion of white blood cells: CD45, specific for white blood cells (22), ensured that only CTCs were counted by excluding white blood cells from the analysis. vi) Automation and sensitivity: The automated CELLSEARCH® system minimized human error and enhanced result reproducibility. It is sensitive and capable of detecting a single CTC in 7.5 ml of blood.

Determination of experimental results. The Cell Search system was used to perform CTC detection. The scanning results obtained from the CellTracks Analyzer II scanning system were then analyzed, and the cells with immunofluorescent staining results showing EpCAM-positive, CK-positive, DAPI-positive and CD45-negative with intact cell membrane and nucleus staining were defined as CTCs. The count of CTCs was recorded, and CTCs ≥1/7.5 ml was defined as CTC-positive. CTC negative indicates the absence of circulating tumor cells in blood samples.

Observational indicators. The relationship between CTCs and sex, age, smoking history, alcohol consumption history, pathological type, tumor site, TNM staging, non-operative

Table I. Basic clinical information of 56 patients.

Pathological features	Number of cases, n (%)
Sex	
Male	52 (92.86)
Female	4 (7.14)
Age	
<50	9 (16.07)
50-60	14 (25.00)
>60	33 (58.93)
Tumor type	
Oropharyngeal carcinoma	5 (8.94)
Oral carcinoma	9 (16.07)
Laryngeal carcinoma	16 (28.57)
Hypopharyngeal carcinoma	13 (23.21)
Nasopharyngeal carcinoma	7 (12.50)
Carcinoma of the nasal cavity and sinuses	6 (10.71)
Pathological type	
Squamous cell carcinoma	52 (92.86)
Non-squamous cell carcinoma	4 (7.14)
Smoking history	
Yes	38 ()
No	18 ()
Drinking history	
Yes	34 (67.86)
No	22 (32.14)
TNM stage	
I + II	16 (28.57)
III	13 (23.22)
IV	27 (48.21)
Surgery	
With	41 (73.21)
Without	15 (26.79)
Non-operative management	
Radiotherapy	21 (37.50)
Chemoradiotherapy	19 (33.93)
None	16 (28.57)

management and whether the patient underwent surgical treatment were analyzed. Furthermore, the impact of CTCs on patient prognosis were examined. Age was categorized into three levels: i) <50; ii) 50-60; and iii) >60 for analysis.

Patients who had not undergone surgery, that is, after comprehensive discussion by a multidisciplinary team, choose to receive non-surgical treatment plans, mainly including radiotherapy or combined chemoradiotherapy. Among these patients, the majority refused surgical treatment due to personal preference, hoping to preserve laryngeal function.

Statistical analysis. This study used SPSS 20.0 software (IBM Corp.) for data analysis. Count data are expressed in the form of n (%). The χ^2 test was used to compare the distribution differences in clinical and pathological characteristics between CTC-positive and CTC-negative patients. Firstly, the patient's outcome of death was defined: Overall survival (OS) was defined as the time from diagnosis to death from any cause, and Kaplan-Meier estimation and log-rank test were used to estimate and compare survival rates between different variables. The impact factors on overall survival were analyzed using univariate and multivariate Cox proportional hazards regression models. $P<0.05$ was considered to indicate a statistically significant difference. The experiments were performed in triplicate.

Results

Basic pathological features of 56 patients. The total 56 patients included 52 males and 4 females, with a median age of 61.5 years (range, 42-75). Stage I + II, III and IV HNC were found in 16, 13 and 27 cases, respectively. All patients were confirmed by histopathology, including 52 cases of SCC and 4 cases of non-squamous cell carcinoma, as presented in Table I.

Detection of CTCs. Blood samples provided by all 56 patients reached a volume of 10 ml without any apparent hemolysis or clotting phenomena, meeting the quality requirements for the assay kit, resulting in a sample qualification rate of 100%. The detection outcomes from the CELLSEARCH® system revealed that out of the 56 patients, 14 individuals exhibited the presence of CTCs with a count of ≥1/7.5 ml, indicating a positivity rate of 25%. The median CTC count stood at 2 (range, 1-32). CTCs, identified by the presence of cytokeratin (CK+) and DAPI positivity (DAPI+), while being negative for the hematopoietic marker CD45 (Fig. 1). Non-CTCs, which are negative for CK (CK-), positive for DAPI (DAPI+), and positive for CD45 were also identified.

Comparison of basic pathological features between CTC-positive and CTC-negative patients. Table II provides a comparative analysis of various basic pathological features between patients who tested positive for CTCs and those who tested negative. When comparing CTC-positive patients with CTC-negative patients, no statistically significant differences were observed in terms of age, sex, smoking/alcohol history (present or absent), tumor location, pathological type, TNM staging and whether surgical treatment was received (all $P>0.05$). The TNM staging was divided into stages I + II, III and IV. The CTC-positive group had 3 (21.43%) in stages I + II, 5 (35.71%) in stage III and 6 (42.86%) in stage IV. The CTC-negative group had 13 (30.95%) in stages I + II, 8 (19.05%) in stage III and 21 (50.00%) in stage IV. The difference in TNM stage distribution was not statistically significant ($\chi^2=1.701$; $P=0.427$). In terms of non-operative management, the CTC-positive group had fewer patients undergoing radiotherapy [3 (21.43%)] and chemoradiotherapy [4 (28.57%)], with a larger proportion not receiving any treatment [7 (50.00%)]. The CTC-negative group had a higher number of patients undergoing radiotherapy [20 (47.62%)] and chemoradiotherapy [15 (35.71%)], with fewer not receiving any treatment [7 (16.67%)]. The difference in non-operative management was significant ($\chi^2=6.578$; $P=0.037$).

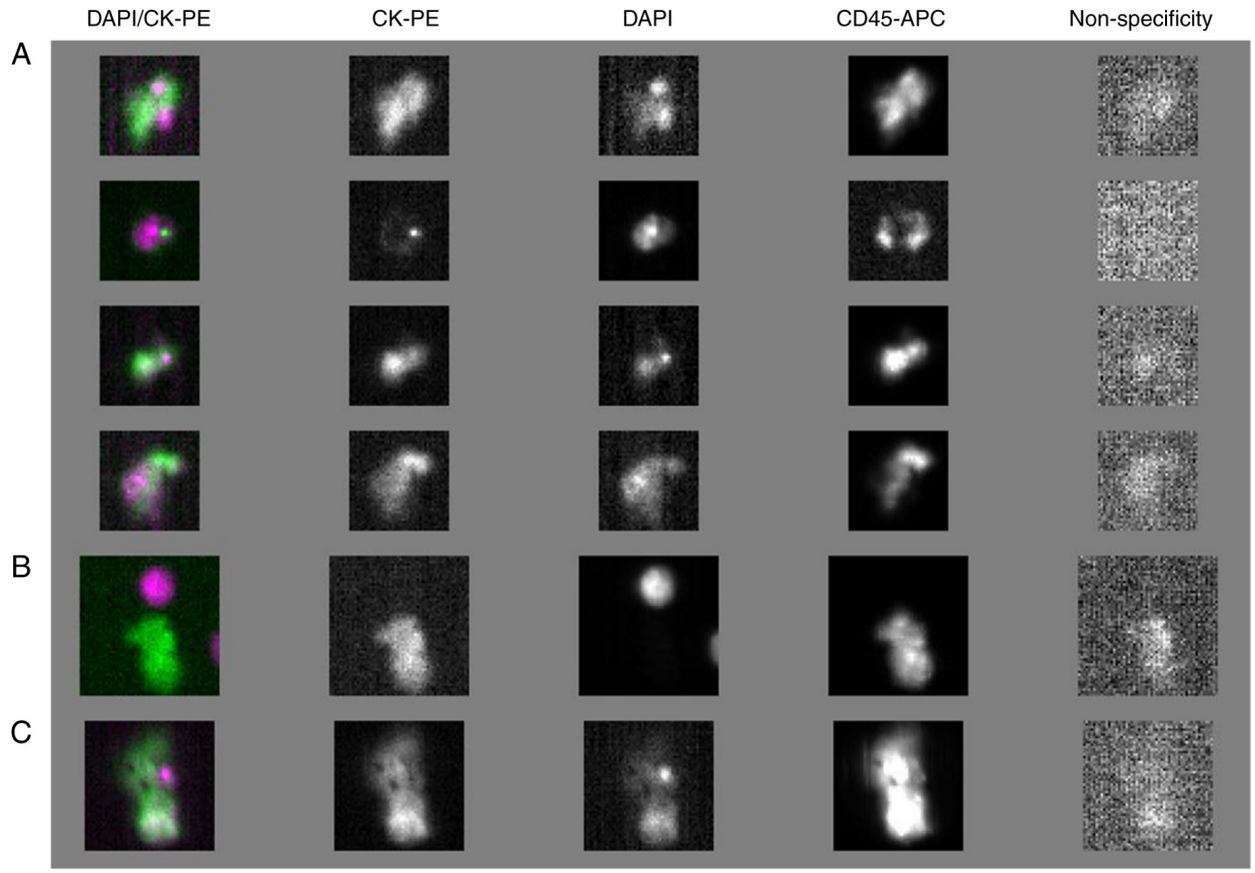

Figure 1. Analytical diagram of the determination of CTC detection results. (A) CTC, CK^+, $DAPI^+$, $CD45^-$; (B) Non-CTC, CK^-, $DAPI^+$, $CD45^+$; (C) Non-CTC: non-specific staining. CTCs, circulating tumor cells; CK, cytokeratin. The magnification, x40.

Comparison of survival rates between CTC-positive and CTC-negative patients. The Kaplan-Meier survival analysis results indicated that the survival rate of CTC-negative patients is higher compared with that of CTC-positive patients, with a statistically significant difference (P=0.0211), as shown in Fig. 2.

Univariate and multivariate analyses of prognosis in patients with HNC. The univariate Cox regression analysis indicated that factors influencing the overall survival of patients include CTCs (HR=1.373; 95% CI, 1.189-1.586; P<0.001), surgical treatment (HR=0.294; 95% CI, 0.124-0.697; P=0.005) and non-operative management (HR=0.404; 95% CI, 0.217-0.752; P=0.004), demonstrating statistical significance for prognosis (P<0.05) (Table III). Subsequently, the multivariate Cox regression analysis revealed that CTCs (HR=1.274; 95% CI, 1.119-1.451; P<0.001) and non-operative management (HR=0.268; 95% CI, 0.119-0.606; P=0.002) independently impact the prognosis of patients with HNC (Table IV).

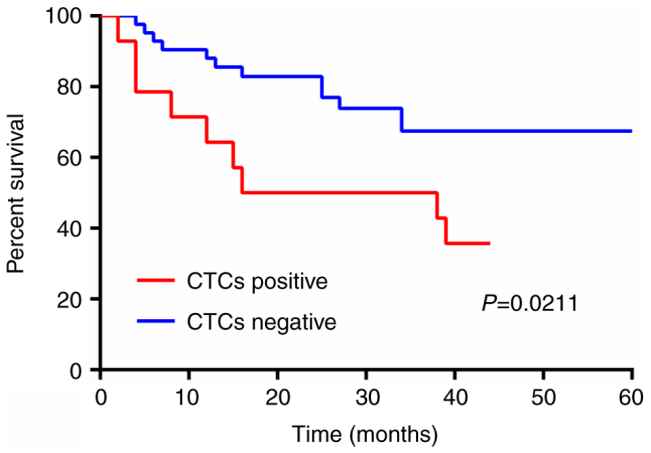

Figure 2. Relationship between CTCs and overall survival in patients, Kaplan-Meier survival analysis of the relationship between CTCs and OS showed that 21 of the 56 patients died, and the median OS of CTC-positive patients was 27 months, a value not reached by CTC-negative patients. CTCs, circulating tumor cells; OS, overall survival.

Discussion

As an emerging 'liquid biopsy' technique, CTC detection has the advantages of simple, real-time, non-invasive and convenient sampling, which provides a novel and simpler tool for real-time monitoring of tumor changes during treatment (14). CTCs, as one of the hot topics in the field of oncology research, have sparked significant interest among numerous researchers. Previous studies have demonstrated that based on the physical characteristics of tumors and the expression or functional features of biomarkers, different methods can be employed to identify CTCs (23,24). CTCs can serve as a biomarker for various stages of disease development and progression. First, CTCs can potentially be detected in the early stages of cancer,

Table II. Comparison of basic pathological features between CTC-positive and CTC-negative patients.

Pathological features	CTCs, n (%)		χ^2	P-value
	Positive (n=14)	Negative (n=42)		
Sex			1.436	0.231
Male	12 (85.71)	40 (95.24)		
Female	2 (14.29)	2 (5.00)		
Age			0.223	0.894
<50	2 (14.29)	7 (16.67)		
50-60	3 (21.43)	11 (26.19)		
>60	9 (64.29)	24 (57.14)		
Tumor type			7.235	0.204
Oropharyngeal carcinoma	3 (21.43)	2 (4.76)		
Oral carcinoma	2 (14.29)	7 (16.67)		
Laryngeal carcinoma	4 (28.57)	12 (28.57)		
Hypopharyngeal carcinoma	2 (14.29)	11 (26.19)		
Nasopharyngeal carcinoma	3 (21.43)	4 (9.52)		
Carcinoma of the nasal cavity and sinuses	0 (0.00)	6 (14.29)		
Pathological type			1.436	0.281
Squamous cell carcinoma	14 (100.00)	38 (90.48)		
Non-squamous cell carcinoma	0 (0.00)	4 (9.52)		
Smoking history			0.109	0.741
Yes	9 (64.29)	29 (69.05)		
No	5 (35.71)	13 (30.95)		
Drinking history			0.898	0.343
Yes	7 (50.00)	27 (64.29)		
No	7 (50.00)	15 (35.71)		
TNM stage			1.701	0.427
I-II	3 (21.43)	13 (30.95)		
III	5 (35.71)	8 (19.05)		
IV	6 (42.86)	21 (50.00)		
Surgical treatment			1.487	0.223
With	12 (85.71)	29 (69.05)		
Without	2 (14.29)	13 (30.95)		
Non-operative management			6.578	0.037
Radiotherapy	3 (21.43)	20 (47.62)		
Chemoradiotherapy	4 (28.57)	15 (35.71)		
None	7 (50.00)	7 (16.67)		

although they are generally rarer in the early stages compared with advanced stages. Second, the presence and quantity of CTCs may increase as the disease progresses, reflecting the growth and potential spread of the tumor. Third, CTC levels can be monitored during treatment to assess response. A decrease in CTCs may indicate effective treatment, while an increase might suggest treatment resistance or disease progression (25,26). Fourth, elevated levels of CTCs can be associated with a higher risk of recurrence or the presence of metastatic disease, as these cells can colonize distant sites (18). At present, the CELLSEARCH® system is the first automated and standardized CTC detection system approved by the U.S. Federal Drug Administration to detect CTCs by identifying epithelial markers EpCAM (positive selection) and leukocyte CD45 (negative selection) using immunomagnetic separation technology (27). The quantity of CTCs can serve as an indicator for predicting the prognosis of metastatic malignant tumors, thus playing an increasingly crucial role in the course of cancer treatment (28-30).

Research has demonstrated the detection of CTCs in peripheral blood of patients with HNC (31,32). Among the 56 patients included in the present study, 14 patients tested positive for CTCs, resulting in a positivity rate of 25%. This finding also indicated the presence of CTCs in the peripheral blood of patients with HNC. Additionally, there are reports indicating that CTCs are more easily

Table III. Univariate survival analysis of overall survival.

Pathological features	β	SEM	P-value	HR	95% CI
Sex	-0.359	0.744	0.630	0.699	0.162-3.005
Age	0.020	0.026	0.444	1.020	0.970-1.073
CTCs	0.317	0.074	<0.001	1.373	1.189-1.586
Tumor type	-0.243	0.157	0.122	0.784	0.576-1.067
Pathological type	-0.675	0.746	0.366	0.509	0.118-2.198
Smoking history	0.163	0.464	0.725	1.177	0.474-2.924
Drinking history	0.238	0.464	0.607	1.269	0.512-3.148
TNM stage	0.057	00.257	0.825	1.059	0.640-1.752
Surgical treatment	-1.224	0.440	0.005	0.294	0.124-0.697
Non-operative management	-0.907	0.317	0.004	0.404	0.217-0.752

HR, hazard ratio; CI, confidence interval; CTCs, circulating tumor cells; TNM, tumor node metastasis.

Table IV. Multivariate survival analysis of overall survival.

Pathological features	β	SEM	P-value	HR	95% CI
CTCs	0.242	0.066	<0.001	1.274	1.119-1.451
Surgical treatment	-0.519	0.484	0.284	0.595	0.230-1.538
Non-operative management	-1.316	0.416	0.002	0.268	0.119-0.606

HR, hazard ratio; CI, confidence interval; CTCs, circulating tumor cells.

detectable in the peripheral blood of patients with advanced stage IV disease and a high tumor burden (33,34). In addition, Buglione *et al* (33) observed that CTCs are more easily found in oropharynx, hypopharynx and paranasal sinuses compared with the oral cavity and nasopharynx. Therefore, this study analyzed the association of CTC level with age, sex, smoking, drinking, pathological type, tumor type, surgical treatment (with vs. without), TNM stage and other pathological features. It found that these clinical characteristic parameters are not associated with the level of CTCs, which is consistent with the previous results (35,36). However, non-operative management (radiotherapy or chemoradiotherapy) exerts a significant effect on the levels of CTCs in patients with HNC in this study (33). Theoretically, the impact of unresected primary tumors on the results of CTC detection is multifaceted, encompassing aspects such as CTC count, heterogeneity, epithelial-mesenchymal transition subtypes, PD-L1 expression, *in vitro* culture conditions, challenges in detection technology and clinical application efficacy (37). The unresected primary tumor may release a greater number of CTCs into the bloodstream, thereby affecting the enumeration and detection outcomes of CTCs (37). However, the present study found that patients who did not undergo surgery exhibited lower levels of CTCs, which may suggest that these patients have tumors at a lower stage. At present, only a few published studies have investigated the effects of radiotherapy on CTCs (34,38-41). In head and neck squamous cell carcinoma (HNSCC), Buglione *et al* (33) studied the role of CTCs in patients who only received radiotherapy, and found a lower sensitivity (30%) in patients receiving radiotherapy compared with those receiving chemotherapy. In addition, radiotherapy combined with chemotherapy has been shown to reduce the CTC count in HNSCC and prostate cancer patients (42-45). Due to the association between the quantity and characteristics of CTCs and the prognosis of cancer patients, it was hypothesized that the detection results of circulating tumor cells can serve as crucial indicators for evaluating patient prognosis and survival outcomes.

In the present study, through Kaplan-Meier survival analysis, it was revealed that the OS of patients testing positive for CTCs exhibited a notably shorter duration in comparison to their CTC-negative counterparts. Tinhofer *et al* (46) revealed the association of CTCs with disease-free survival (DFS) and OS in patients with oropharyngeal cancer, indicating that the presence of CTCs in these patients indicates an adverse prognosis. The role of CTC detection in evaluating the efficacy and prognosis of HNC is still under study, with limited research indicating a certain role played by CTCs in evaluating prognosis in HNC. For example, Jatana *et al* (47) found that CTCs are related to DFS and that patients with a high number of CTCs detected have a poor prognosis. In addition to the prognostic information provided by baseline CTCs, changes in the count of CTCs during treatment can also provide additional information: The persistence of CTCs during treatment can help identify tumors that do not respond to treatment, allowing for timely adjustment of the treatment protocol (48). Through the application of multivariable Cox regression analysis, the

present study conclusively demonstrated that CTCs (HR=1.274; 95% CI, 1.119-1.451; P<0.001) and non-operative management (HR=0.268; 95% CI, 0.119-0.606; P=0.002) stood out as independent factors exerting a significant impact on the prognosis of patients with HNC. Furthermore, this investigation not only underscored the critical role of CTCs as a valuable clinical indicator for prognostic evaluation but also provided compelling evidence to support their incorporation in prognostic assessments.

Additionally, molecular therapies for patients with HNC have been an area of significant advancement. These therapies target specific molecular pathways or genetic alterations that contribute to cancer growth and progression. The following are some molecular therapy models and approaches that are being used or explored for HNC: i) EGFR inhibitors (49); ii) PI3K/AKT/mTOR pathway inhibitors: Drugs such as mTOR inhibitors everolimus and ridaforolimus have been explored in clinical trials (50); iii) immune checkpoint inhibitors, which include PD-1/PD-L1 inhibitors (such as pembrolizumab and nivolumab) and CTLA-4 inhibitors (such as ipilimumab), have shown promise in treating HNC, particularly in patients with advanced disease (51); iv) fibroblast growth factor receptor inhibitors (52); v) VEGF inhibitors: Bevacizumab, a monoclonal antibody against VEGF, has been used to target angiogenesis in HNC (53); and vi) hedgehog pathway inhibitors: Smoothened inhibitors such as vismodegib have been studied for HNC, as the hedgehog pathway is implicated in tumor growth (54).

Use of molecular therapies in HNC is complex and requires careful patient selection based on tumor molecular profiling. A number of these therapies are part of ongoing research and their integration into standard care depends on the results of clinical trials and an understanding of their safety and efficacy. Precision medicine, which involves tailoring treatments to the molecular profile of a tumor, is a growing field in oncology (55). The success of molecular therapies in HNC will likely lead to more personalized treatment strategies in the future (55). It should be noted that although the CELLSEARCH® system is the only CTC detection system approved by regulatory authorities in the United States and China, it has standardized operating procedures and good comparability of results, and CELLSEARCH® technology has been used for the detection of various types of cancer, such as breast cancer, renal cancer, lung cancer, ovarian cancer and brain cancer (56). However, the diagnostic cutoff values for CTC in different cancers are still unclear and not comparable at present (57).

The present study has several limitations. While the potential significance of CTC detection in the early diagnosis, treatment monitoring, and prognosis assessment of HNC is under scrutiny, and current research findings exhibit inconsistencies with previous reports, significantly hindering the widespread adoption of CTC detection in large-scale clinical settings. These discrepancies may stem from the intricate and varied biological and clinical characteristics displayed by the anatomical origins of HNC (58,59). Owing to these divergent characteristics, prior studies have often been confined to relatively modest patient cohorts, further exacerbated by substantial heterogeneity among these patient groups, inevitably amplifying uncertainty and rendering comparisons between research outcomes challenging. Moreover, the relatively limited sample sizes utilized in studies have somewhat diminished the statistical robustness of research findings, casting doubts on the reliability of the conclusions drawn. Notably, research on the association between CTC count and treatment response in HNC is still in its nascent stages, with existing studies lacking the requisite depth and breadth to underpin clinical decision-making (59). Due to these circumstances, there exists a pressing need for larger-scale, prospective clinical investigations to comprehensively explore the utility of CTC detection in monitoring treatment efficacy and evaluating prognosis across distinct clinical subtypes of patients with HNC (60). Through enlarging sample sizes, refining research methodologies and fully acknowledging the biological and clinical disparities inherent in HNC, we endeavor to provide sturdier and dependable evidence to bolster the integration of CTC detection in clinical practice for HNC. Due to the relatively small sample size at present, this study can be considered an initial research attempt. Looking forward, we hope to increase the sample size in future research work to evaluate the effectiveness of this technology. Additionally, as a retrospective study, we do not have data on circulating tumor DNA and therefore, regrettably, the present study lacked the relevant data to report. Detection of circulating tumor DNA will be the future research direction.

The present study revealed that patients with elevated levels of CTCs had a poorer prognosis. Both univariate and multivariate analyses demonstrated that CTC levels were one of the independent factors influencing the prognosis of patients with HNC. The detection of CTCs holds potential value in the diagnosis and prognosis assessment of patients with HNC. By monitoring CTC levels, physicians can gain a greater understanding of the disease progression in patients, thereby providing a robust basis for devising personalized treatment plans.

Acknowledgements

Not applicable.

Funding

This study was funded by the Beijing Natural Science Foundation (grant no. 7122039).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HYL contributed to the conception and design of the study. PDL, FL and TW collected and analyzed data. HYL wrote and revised the manuscript. HYL, PDL, FL and TW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study protocol was in accordance with the Declaration of Helsinki of the World Medical Association. The study was approved by the ethical committee of the Beijing

Tongren Hospital, Capital Medical University (approval no. 23-B4-03). Informed consent was waived for this study because the research was retrospective and conducted on anonymized data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Mody MD, Rocco JW, Yom SS, Haddad RI and Saba NF: Head and neck cancer. Lancet 398: 2289-2299, 2021.
2. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE and Grandis JR: Head and neck squamous cell carcinoma. Nat Rev Dis Primers 6: 92, 2020.
3. Di Credico G, Polesel J, Dal Maso L, Pauli F, Torelli N, Luce D, Radoï L, Matsuo K, Serraino D, Brennan P, *et al*: Alcohol drinking and head and neck cancer risk: The joint effect of intensity and duration. Br J Cancer 123: 1456-1463, 2020.
4. Gupta I, Ghabreau L, Al-Thawadi H, Yasmeen A, Vranic S, Al Moustafa AE and Malki MI: Co-incidence of human papillomaviruses and Epstein-barr virus is associated with high to intermediate tumor grade in human head and neck cancer in syria. Front Oncol 10: 1016, 2020.
5. Mourad M, Jetmore T, Jategaonkar AA, Moubayed S, Moshier E and Urken ML: Epidemiological trends of head and neck cancer in the united states: A SEER population study. J Oral Maxillofac Surg 75: 2562-2572, 2017.
6. Economopoulou P, Kotsantis I, Kyrodimos E, Lianidou ES and Psyrri A: Liquid biopsy: An emerging prognostic and predictive tool in Head and Neck Squamous Cell Carcinoma (HNSCC). Focus on Circulating Tumor Cells (CTCs). Oral Oncol 74: 83-89, 2017.
7. Takes RP, Rinaldo A, Silver CE, Haigentz M Jr, Woolgar JA, Triantafyllou A, Mondin V, Paccagnella D, de Bree R, Shaha AR, *et al*: Distant metastases from head and neck squamous cell carcinoma. Part I. Basic aspects. Oral Oncol 48: 775-779, 2012.
8. Kopczyński P and Flieger R: Tumor markers used in the diagnosis, monitoring, treatment, and prognosis head and neck cancer. Pol Merkur Lekarski 35: 37-38, 2013 (In Polish).
9. Eskander A, Irish J, Groome PA, Freeman J, Gullane P, Gilbert R, Hall SF, Urbach DR and Goldstein DP: Volume-outcome relationships for head and neck cancer surgery in a universal health care system. Laryngoscope 124: 2081-2088, 2014.
10. Leipzig B, Zellmer JE and Klug D: The role of endoscopy in evaluating patients with head and neck cancer. A multi-institutional prospective study. Arch Otolaryngol 111: 589-594, 1985.
11. Chung CS, Lo WC, Wen MH, Hsieh CH, Lin YC and Liao LJ: Long term outcome of routine Image-enhanced endoscopy in newly diagnosed head and neck cancer: A prospective study of 145 patients. Sci Rep 6: 29573, 2016.
12. Pantel K and Speicher MR: The biology of circulating tumor cells. Oncogene 35: 1216-1224, 2016.
13. Novel Biomarkers in the Continuum of Breast Cancer. Anticancer Res 36: 3224, 2016.
14. Shen Z, Wu A and Chen X: Current detection technologies for circulating tumor cells. Chem Soci Rev 46: 2038-2056, 2017.
15. Bidard FC, Proudhon C and Pierga JY: Circulating tumor cells in breast cancer. Mol Oncol 10: 418-430, 2016.
16. Pantel K, Hille C and Scher HI: Circulating tumor cells in prostate cancer: From discovery to clinical utility. Clin Chem 65: 87-99, 2019.
17. Jia S, Zhang R, Li Z and Li J: Clinical and biological significance of circulating tumor cells, circulating tumor DNA, and exosomes as biomarkers in colorectal cancer. Oncotarget 8: 55632-55645, 2017.
18. Tong M, Luo S, Gu L, Wang X, Zhang Z, Liang C, Huang H, Lin Y and Huang J: SIMarker: Cellular similarity detection and its application to diagnosis and prognosis of liver cancer. Comput Biol Med 171: 108113, 2024.
19. Ramirez JM, Fehm T, Orsini M, Cayrefourcq L, Maudelonde T, Pantel K and Alix-Panabières C: Prognostic relevance of viable circulating tumor cells detected by EPISPOT in metastatic breast cancer patients. Clin Chem 60: 214-221, 2014.
20. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y and Zhou J: Circulating tumor cells: Biology and clinical significance. Signal Transduct Target Ther 6: 404, 2021.
21. Coumans F and Terstappen L: Detection and characterization of circulating tumor cells by the cellsearch approach. Methods Mol Biol 1347: 263-278, 2015.
22. Rheinländer A, Schraven B and Bommhardt U: CD45 in human physiology and clinical medicine. Immunol Lett 196: 22-32, 2018.
23. Alix-Panabières C and Pantel K: Challenges in circulating tumour cell research. Nat Rev Cancer 14: 623-631, 2014.
24. Alix-Panabières C and Pantel K: Circulating tumor cells: Liquid biopsy of cancer. Clin Chem 59: 110-118, 2013.
25. Deng Z, Wu S, Wang Y and Shi D: Circulating tumor cell isolation for cancer diagnosis and prognosis. EBioMedicine 83: 104237, 2022.
26. Pereira-Veiga T, Schneegans S, Pantel K and Wikman H: Circulating tumor cell-blood cell crosstalk: Biology and clinical relevance. Cell Rep 40: 111298, 2022.
27. Yu M, Stott S, Toner M, Maheswaran S and Haber DA: Circulating tumor cells: Approaches to isolation and characterization. J Cell Biol 192: 373-382, 2011.
28. Park CH, Lee JI, Sung J, Choi S and Ko KP: A flow visualization model of duodenogastric reflux after esophagectomy with gastric interposition. J Cardiothorac Surg 8: 192, 2013.
29. Zhang Z, Xiao Y, Zhao J, Chen M, Xu Y, Zhong W, Xing J and Wang M: Relationship between circulating tumour cell count and prognosis following chemotherapy in patients with advanced non-small-cell lung cancer. Respirology 21: 519-525, 2016.
30. Nieva J, Wendel M, Luttgen MS, Marrinucci D, Bazhenova L, Kolatkar A, Santala R, Whittenberger B, Burke J, Torrey M, *et al*: High-definition imaging of circulating tumor cells and associated cellular events in non-small cell lung cancer patients: A longitudinal analysis. Phys Biol 9: 016004, 2012.
31. Nichols AC, Lowes LE, Szeto CC, Basmaji J, Dhaliwal S, Chapeskie C, Todorovic B, Read N, Venkatesan V, Hammond A, *et al*: Detection of circulating tumor cells in advanced head and neck cancer using the CellSearch system. Head Neck 34: 1440-1444, 2012.
32. Bozec A, Ilie M, Dassonville O, Long E, Poissonnet G, Santini J, Chamorey E, Ettaiche M, Chauvière D, Peyrade F, *et al*: Significance of circulating tumor cell detection using the CellSearch system in patients with locally advanced head and neck squamous cell carcinoma. Eur Arch Otorhinolaryngol 270: 2745-2749, 2013.
33. Buglione M, Grisanti S, Almici C, Mangoni M, Polli C, Consoli F, Verardi R, Costa L, Paiar F, Pasinetti N, *et al*: Circulating tumour cells in locally advanced head and neck cancer: Preliminary report about their possible role in predicting response to non-surgical treatment and survival. Eur J Cancer 48: 3019-3026, 2012.
34. Guney K, Yoldas B, Ozbilim G, Derin AT, Sarihan S and Balkan E: Detection of micrometastatic tumor cells in head and neck squamous cell carcinoma. A possible predictor of recurrences? Saudi Med J 28: 216-220, 2007.
35. Li H, Li B, Pan Y, Zhang Y, Xiang J, Zhang Y, Sun Y, Yu X, He W and Hu H: Preoperative folate receptor-positive circulating tumor cell level is a prognostic factor of long term outcome in non-small cell lung cancer patients. Front Oncol 10: 621435, 2020.
36. Bertamini L and Oliva S: High levels of circulating tumor plasma cells as a key hallmark of aggressive disease in Transplant-Eligible patients with newly diagnosed multiple myeloma. J Clin Oncol 40: 3120-3131, 2022.
37. Ju L, Yang J, Zhai C, Chai S, Dong Z and Li M: Survival, Chemotherapy and Chemosensitivity Predicted by CTC Cultured In Vitro of SCLC Patients. Front Oncol 11: 683318, 2021.
38. Martin OA, Anderson RL, Russell PA, Cox RA, Ivashkevich A, Swierczak A, Doherty JP, Jacobs DH, Smith J, Siva S, *et al*: Mobilization of viable tumor cells into the circulation during radiation therapy. Int J Radiat Oncol Biol Phys 88: 395-403, 2014.
39. Morosin T, Ashford B, Ranson M, Gupta R, Clark J, Iyer NG and Spring K: Circulating tumour cells in regionally metastatic cutaneous squamous cell carcinoma: A pilot study. Oncotarget 7: 47111-47115, 2016.
40. Balasubramanian P, Lang JC, Jatana KR, Miller B, Ozer E, Old M, Schuller DE, Agrawal A, Teknos TN, Summers TA Jr, *et al*: Multiparameter analysis, including EMT markers, on negatively enriched blood samples from patients with squamous cell carcinoma of the head and neck. PLoS One 7: e42048, 2012.

41. Dorsey JF, Kao GD, MacArthur KM, Ju M, Steinmetz D, Wileyto EP, Simone CB II and Hahn SM: Tracking viable circulating tumor cells (CTCs) in the peripheral blood of non-small cell lung cancer (NSCLC) patients undergoing definitive radiation therapy: Pilot study results. Cancer 121: 139-149, 2015.
42. Jatana KR, Balasubramanian P, Lang JC, Yang L, Jatana CA, White E, Agrawal A, Ozer E, Schuller DE, Teknos TN and Chalmers JJ: Significance of circulating tumor cells in patients with squamous cell carcinoma of the head and neck: Initial results. Arch Otolaryngol Head Neck Surg 136: 1274-1279, 2010.
43. Winter SC, Stephenson SA, Subramaniam SK, Paleri V, Ha K, Marnane C, Krishnan S and Rees G: Long term survival following the detection of circulating tumour cells in head and neck squamous cell carcinoma. BMC Cancer 9: 424, 2009.
44. Jatana KR, Balasubramanian P, McMullen KP, Lang JC, Teknos TN and Chalmers JJ: Effect of surgical intervention on circulating tumor cells in patients with squamous cell carcinoma of the head and neck using a negative enrichment technology. Head Neck 38: 1799-1803, 2016.
45. Gao W, Li JZ, Ho WK, Chan JY and Wong TS: Biomarkers for use in monitoring responses of nasopharyngeal carcinoma cells to ionizing radiation. Sensors (Basel) 12: 8832-8846, 2012.
46. Tinhofer I, Hristozova T, Stromberger C, Keilhoiz U and Budach V: Monitoring of circulating tumor cells and their expression of EGFR/phospho-EGFR during combined radiotherapy regimens in locally advanced squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 83: e685-e690, 2012.
47. Jatana KR, Lang JC and Chalmers JJ: Identification of circulating tumor cells: A prognostic marker in squamous cell carcinoma of the head and neck? Future Oncol 7: 481-484, 2011.
48. Schochter F, Friedl TWP, deGregorio A, Krause S, Huober J, Rack B and Janni W: Are Circulating tumor cells (CTCs) ready for clinical use in breast cancer? An overview of completed and ongoing trials using CTCs for clinical treatment decisions. Cells 8: 1412, 2019.
49. Kyrodimos E, Chrysovergis A, Mastronikolis N, Tsiambas E, Ragos V, Roukas D, Fotiades P and Papanikolaou V: Targeting EGFR in nasopharyngeal carcinoma. J BUON 26: 759-761, 2021.
50. Aguayo F, Perez-Dominguez F and Osorio JC: PI3K/AKT/mTOR signaling pathway in HPV-Driven head and neck carcinogenesis: Therapeutic implications. Biology (Basel) 12: 672, 2023.
51. O'Meara CH and Jafri Z: Immune checkpoint inhibitors, Small-molecule immunotherapies and the emerging role of neutrophil extracellular traps in therapeutic strategies for head and neck cancer. Int J Mol Sci 24: 11695, 2023.
52. Bao Y, Gabrielpillai J, Dietrich J, Zarbl R, Strieth S, Schröck F and Dietrich D: Fibroblast growth factor (FGF), FGF receptor (FGFR), and cyclin D1 (CCND1) DNA methylation in head and neck squamous cell carcinomas is associated with transcriptional activity, gene amplification, human papillomavirus (HPV) status, and sensitivity to tyrosine kinase inhibitors. Clin Epigenetics 13: 228, 2021.
53. Khadela A, Shah Y, Mistry P, Mansuri M, Sureja D and Bodiwala K: A review of efficacy and safety of cetuximab and bevacizumab-based monoclonal antibodies in head and neck cancer. Med Oncol 40: 66, 2022.
54. Cierpikowski P, Leszczyszyn A and Bar J: The role of hedgehog signaling pathway in head and neck squamous cell carcinoma. Cells 12: 2083, 2023.
55. Tsimberidou AM, Fountzilas E, Nikanjam M and Kurzrock R: Review of precision cancer medicine: Evolution of the treatment paradigm. Cancer Treat Rev 86: 102019, 2020.
56. de Wit S, van Dalum G and Terstappen LW: Detection of circulating tumor cells. Scientifica (Cairo) 2014: 819362, 2014.
57. Hoeppener AE, Swennenhuis JF and Terstappen LW: Immunomagnetic separation technologies. Recent Results Cancer Res 195: 43-58, 2012.
58. Hanna GJ, Patel N, Tedla SG, Baugnon KL, Aiken A and Agrawal N: Personalizing surveillance in head and neck cancer. Am Soc Clin Oncol Educ Book 43: e389718, 2023.
59. Huang X, Duijf PHG, Sriram S, Perera G, Vasani S, Kenny L, Leo P and Punyadeera C: Circulating tumour DNA alterations: Emerging biomarker in head and neck squamous cell carcinoma. J Biomed Sci 30: 65, 2023.
60. Tsutsuyama M and Nakanishi H: Detection of circulating tumor cells in drainage venous blood from colorectal cancer patients using a new filtration and cytology-based automated platform. PLoS One 14: e0212221, 2019.