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ORIGINAL RESEARCH

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Circulating tumor cell analysis in locally advanced and metastatic squamous cell carcinoma of the head and neck

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Abstract

Background: Circulating tumors cells (CTCs) are considered an early step towards metastasis and have been linked to poor prognosis in several types of cancer. CTCs in squamous cell carcinoma of the head and neck (SCCHN) have an unclear role.

Methods: In this prospective study, patients with locally advanced or metastatic SCCHN had CTC counts assessed before starting systemic treatment using the CellSearch System. Select cases also had sequential CTC evaluation. Presence of CTCs was correlated with patient characteristics and outcomes.

Results: Forty-eight patients enrolled, and 36 had evaluable clinical data and baseline CTC counts. Twenty-five patients had locally advanced disease (LAD) and 11 had metastatic disease. ≥1 CTCs were detected in six patients with LAD (24%) and four with metastatic disease (36%). On univariate analysis, smoking was associated with CTCs.

Conclusion: CTCs are not associated with prognosis in patients with LAD and metastatic disease; however, they are present in this patient population, and ≥1 CTCs is associated with a history of smoking.

Level of evidence: 1b; individual prospective cohort study.

KEYWORDS

circulating tumor cells, head and neck cancer, smoking

1 | INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) accounts for approximately 5% of newly diagnosed cancer cases, equaling roughly 644 000 cases and over 350 000 cancer deaths worldwide each year.[1,2] Patients typically present with locoregionally advanced disease (LAD), and prognosis is associated with age, human papillomavirus (HPV) status, smoking or alcohol history, and lymph node involvement.[3] Despite advances in the treatment for these patients, long-term disease-free and overall survival remains poor—approximately 40% to 60% of patients develop local recurrences and 20% to 30% are diagnosed with metastatic disease.[1,2]

Circulating tumor cells (CTCs) and their clusters, circulating tumor microemboli (CTM), (defined as ≥3 CTCs) are detached tumor cells

from the primary or metastatic sites that infiltrate into the lymphatic and circulatory system. In the context of localized disease, CTCs and CTMs are considered an early step towards metastatic spread. When present in peripheral blood, they correlate with poor prognoses in breast, gastric, and prostate cancer.[4-8]

In patients with regionally advanced or metastatic SCCHN, CTCs have been found in 18% to 33% of patients[9,10]; other studies have demonstrated that CTCs are found independently of T-stage and HPV status.[11,12] However, CTCs have an unclear impact on prognosis. Some studies have reported that CTCs correlate with reduced disease-free survival, progression-free survival (PFS) and overall survival (OS),[10,13,14] including one study of 53 patients with recurrent or metastatic SCCHN that found CTCs were associated with lower PFS and OS.[10] Another study of 73 patients with LAD found that a decrease in CTC count during treatment suggested nonprogressive disease.[15] Others have found that CTCs did not correlate to outcomes.[9,16] More research is needed to clarify the prognostic value of CTCs in SCCHN, which is the goal of this study.

In this prospective pilot study, we enrolled 48 patients with locally advanced or metastatic SCCHN and collected peripheral blood samples before starting systemic treatment. Select patients also had sequential blood draws. Patients had samples analyzed by CellSearch, which uses upregulated epithelial cell adhesion molecule (EpCAM) to identify potential CTCs and further stratifies them based on cellular markers. We used this technology as it is established that EpCAM is upregulated in many carcinomas, including SCCHN.[17,18]

2 | METHODS

2.1 | Patient eligibility and demographics

The Institutional Review Board at Dana-Farber/Brigham and Women's Cancer Center approved the research protocol, and all accrued patients signed written informed consent prior to enrollment. Eligible patients had pathologically confirmed SCCHN and locoregionally advanced or distant metastatic (stage III or IV, AJCC seventh edition) disease by imaging, were receiving treatment at Dana-Farber Cancer Institute (DFCI), and had an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less. Patients treated outside of DFCI or without tumor tissue available were excluded from the study.

Prior to enrollment, patients who consented to the study had previously been assigned treatment by their treating oncologists to one of three regimens: sequential induction followed by chemoradiotherapy (CRT), concurrent CRT, or systemic treatment for distant metastatic disease, which were also the three cohorts in this study. Blood samples were collected based on cohort: patients with LAD and metastatic cancer had 7.5 mL of whole blood collected before starting treatment. Some patients also had sequential blood draws throughout treatment and during follow-up. Patient demographics were collected and deidentified, and subjects were followed for PFS and OS. PFS is the time from initiation of treatment to disease progression or loss to follow-up, and OS is the time from initiation of treatment to death or loss to follow-up. Disease burden was assessed using clinical notes and imaging studies taken for standard of treatment.

2.2 | Isolation and enumeration of CTCs

Blood samples were collected before, during, and after treatment. Fresh, unrefrigerated peripheral blood samples were processed within 96 hours of collection using the CellSearch System (Huntingdon Valley, Pennsylvania). Briefly, samples were immunomagnetically enriched for CTCs with anti-EpCAM conjugated magnetic beads and subsequently stained with immunofluorescent antibodies to cytokeratins 8, 18, and 19 and CD45 and a 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain. Carcinoma cells were identified in a semi-automated fashion as cells positive for cytokeratin, negative for CD45, and fulfilling the predefined morphologic criteria of the food and drug administration (FDA)-cleared in vitro diagnostic test. All samples were reviewed by a trained pathologist (A. C. L.). Results were reported in number of CTCs identified per 7.5 mL of whole blood.

2.3 | Statistical analysis

Fisher's exact tests, likelihood ratios, and Mann-Whitney *U* tests were used in univariate analysis to determine factors associated with presence of CTCs. Statistical analyses were performed using SPSS version 24 (IBM; Armonk, NY).

3 | RESULTS

Forty-eight patients were enrolled, but one patient had inaccessible medical records and was not included in analysis. Forty-seven patients (44 men, 3 women), with a median age of 55.5 (24-66), enrolled from October 2011 to October 2015. Of those with known HPV status, 30 patients had HPV (+) disease and 5 had HPV (–) disease. Twenty-three patients had a significant alcohol use history, defined as ≥5 drinks/week, and 20 had a significant smoking history, defined as ≥10 pack-years. Thirty-three patients had LAD, and most received cisplatin or carboplatin with definitive intensity modulated radiation therapy for their initial treatment (n = 31). Fourteen patients had metastatic disease and received varying systemic treatment regimens (Table 1). The median follow-up was 35.5 months for the cohort.

Of the 47 patients, 36 (75%) had a successful CTC assessment prior to the start of treatment and clinical data; 8 patients never underwent CTC draw, 1 patient withdrew consent from the study prior to capturing outcome data, and two patients had incomplete CTC analysis due to poor sample quality and CellSearch failure, respectively. Twenty-five patients had LAD and 11 had metastatic disease. Most patients had primary lesions in the oropharynx (n = 23) and HPV (+) disease (n = 23). In this cohort, 19 patients had a smoking

TABLE 1 Consented patient characteristics

	All patients[a] (%) (n = 47)	Induction arm (%) (n = 15)	CRT arm (%) (n = 18)	Metastatic arm (%) (n = 14)
Median age at blood draw (range), y	55.0 (24-72)	55 (24-66)	52 (30-72)	60.5 (50-68)
Gender				
Male	44 (93.6%)	13 (86.7%)	17 (94.4%)	14 (100.0%)
Female	3 (6.4%)	2 (13.3%)	1 (5.6%)	0 (0.0%)
HPV status[b]				
Positive	30 (85.7%)	12 (92.3%)	10 (90.9%)	8 (72.7%)
Negative	5 (14.3%)	1 (7.7%)	1 (9.1%)	3 (27.3%)
Smoking history[c]				
<10 pack-years	26 (56.5%)	9 (60.0%)	12 (70.6%)	5 (35.7%)
≥10 pack-years	20 (43.5%)	6 (40.0%)	5 (29.4%)	9 (64.3%)
Alcohol history[d]				
≥5 drinks/wk	23 (52.3%)	8 (57.1%)	7 (41.2%)	8 (61.5%)
<5 drinks/wk	21 (47.7%)	6 (42.9%)	10 (58.8%)	5 (38.5%)
Most recent systemic treatment prior to CTC draw				
Cisplatin	19 (40.4%)	3 (20.0%)	15 (83.3%)	1 (7.1%)
Carboplatin	15 (31.9%)	10 (66.7%)	3 (16.7%)	2 (14.3%)
Cetuximab and carboplatin	2 (4.3%)	2 (13.3%)	-	-
Nivolumab	2 (4.3%)	-	-	2 (14.3%)
Pembrolizumab	2 (4.3%)	-	-	2 (14.3%)
EXTREME	1 (2.1%)	-	-	1 (7.1%)
Cetuximab and gemcitabine	1 (2.1%)	-	-	1 (7.1%)
5-FU and leucovorin	1 (2.1%)	-	-	1 (7.1%)
Vinorelbine	1 (2.1%)	-	-	1 (7.1%)
Taxotere	1 (2.1%)	-	-	1 (7.1%)
Gemcitabine	1 (2.1%)	-	-	1 (7.1%)
Cetuximab	1 (2.1%)	-	-	1 (7.1%)
Median follow-up time from study entry (range), mo	35.5 (2-71)	58 (13-71)	39.0 (4-64)	6 (2-20)

Abbreviations: 5-FU, 5-fluorouracil; CRT, concurrent radiotherapy; CTC = circulating tumor cells; HPV, human papillomavirus.
[a]One patient had an inaccessible medical record and was excluded from analysis.
[b]A total of 12 patients (2 patients in induction arm, 7 patients in CRT arm, and 3 patients in metastatic arm) had unknown HPV status.
[c]One patient in the CRT arm had unknown smoking history.
[d]A total of three patients (one patient in induction arm, one patient in CRT arm, and one patient in metastatic arm) had unknown alcohol history.

history <10 pack years, and 19 had <5 alcoholic drinks/week. Baseline CTCs were detected in six patients with LAD and four with metastatic disease and had varying amounts (range = 1-9). Primary location, T-stage, N-stage, disease burden, HPV association, median PFS, and median OS were not associated with ≥1 CTCs. Significant smoking history (P = .002) was associated with ≥1 CTCs—10 patients had detectable CTCs at baseline and 9 had a significant smoking history (Table 2). Of these nine patients, three patients also had a significant alcohol history. Comparing LAD and metastatic SCCHN PFS and OS with CTCs did not demonstrate a significant correlation (Table 3).

Separately, 18 patients had sequential CTC blood draws at baseline and during or after treatment. Eleven patients never had ≥1 CTCs, while seven did. Of the seven patients, three patients had CTCs detected once, while four patients had CTCs detected more than once. Timing of blood draws and number (range: 2-12) varied. Neither median PFS nor OS were significantly associated with CTCs (Table 4). Within this cohort, three patients had CTMs; one patient had CTMs during treatment, and two patients had CTMs before treatment. The two patients who had CTMs before treatment had metastatic disease and CTCs ≥2 at multiple time points. The patients died 2 and 6 months after the baseline draw, respectively. Notably, the former patient had 170 CTCs 4 days before death.

4 | DISCUSSION

We conducted a prospective pilot study investigating the effect of CTCs on PFS and OS in patients with locally advanced or metastatic

TABLE 2 Factors associated with baseline CTCs

	0 CTCs (%) (n = 26)	≥ 1 CTCs (%) (n = 10)	*P* value
Primary tumor location			
Oropharynx	19 (73.1%)	4 (40.0%)	.119
Other	7 (26.9%)	6 (60.0%)	
T staging[a]			
T1	5 (20.8%)	2 (25.0%)	.449
T2	6 (25.0%)	4 (50.0%)	
T3	9 (37.5%)	1 (12.5%)	
T4	4 (16.7%)	1 (12.5%)	
N staging[b]			
<N2b	7 (26.9%)	1 (14.3%)	.652
≥N2b	19 (73.1%)	6 (85.7%)	
Disease burden at time of draw			
LAD	19 (73.1%)	6 (60.0%)	.454
Metastatic disease	7 (26.9%)	4 (40.0%)	
HPV status[c]			
Positive	18 (85.7%)	5 (83.3%)	1
Negative	3 (14.3%)	1 (16.7%)	
Smoking history			
<10 pack-years	18 (69.2%)	1 (10.0%)	.002
≥10 pack-years	8 (30.8%)	9 (90.0%)	
Alcohol history[d]			
<5 drinks/wk	15 (62.5%)	4 (40.0%)	.276
≥5 drinks/wk	9 (37.5%)	6 (60.0%)	
Median OS[e] (range), mo	32.10 (3-72)	30.30 (1-70)	.337
Median PFS[f] (range), mo	28.00 (1-71)	28.00 (1-70)	.614

Abbreviations: CTC, circulating tumor cells, HPV, human papillomavirus; LAD, locally advanced disease; OS, overall survival, PFS, progression-free survival.
[a]Two patients in the "0 CTCs" group and two patients in the "CTCs observed" group had unknown T-staging.
[b]Three patients in the "≥1 CTCs group" had unknown N staging.
[c]Five patients in the "0 CTCs" group and four patients in the "CTCs observed" group had unknown HPV status.
[d]Two patients in the "0 CTCs" group had unknown alcohol history.
[e]PFS is until disease progression or last available follow-up.
[f]OS is until death or last available follow-up.

TABLE 3 Disease burden at time of baseline CTC draw vs PFS and OS

Locally advanced disease	**0 CTCs (n = 19)**	**≥1 CTCs (n = 6)**	***P* value**
Median PFS[a] (range), mo	41.00 (4-71)	38.50 (25-69)	.975
Median OS[b] (range), mo	44.40 (5-72)	40.30 (26-70)	.555
Metastatic disease	**0 CTCs (n = 7)**	**≥1 CTCs (n = 4)**	***P* value**
Median PFS[a] (range), mo	4.00 (1-7)	2.50 (1-19)	.927
Median OS[b] (range), mo	11.13 (3-21)	5.37 (1-19)	.412

Abbreviations: CTC, circulating tumor cells; OS, overall survival; PFS, progression-free survival.
[a]PFS is until disease progression or last available follow-up.
[b]OS is until death or last available follow-up.

TABLE 4 CTCs in patients with sequential draws vs PFS and OS

	0 CTCs (n = 11)	≥1 CTCs (n = 7)	*P* value
Median PFS[a] (range), mo	31.00 (1-71)	13.00 (1-69)	.126
Median OS[b] (range), mo	60.73 (3-72)	13.73 (1-70)	.285

Abbreviations: CTC, circulating tumor cells; OS, overall survival; PFS, progression-free survival.
[a]PFS is until disease progression or last available follow-up.
[b]OS is until death or last available follow-up.

SCCHN. Forty-eight patients enrolled in this study and 36 patients had evaluable CTCs with correlating clinical data. Of the 36 patients, CTCs were detected in six patients with LAD and four patients with metastatic disease (24% and 36%, respectively). A significant smoking history was associated with CTCs on univariate analysis, while tumor stage and other known prognostic markers were not.

CTCs have been studied in various cancers, and these studies suggest that increased CTCs are associated with a poorer prognosis. In breast cancer, CTC-positive patients (≥5 CTCs/7.5 mL) have decreased PFS and OS,[19] and in a study of 216 patients with ovarian cancer, ≥2 CTCs at baseline predicted increased risk for both progression and death.[20] The majority of findings in lung cancer that analyzed CTCs using CellSearch, both in nonsmall cell lung cancer and small cell lung cancer, have found that increased CTCs predict poorer prognosis.[21]

Thus far, few studies have been published about the impact of CTCs in head and neck cancer. One study of 53 patients with recurrent/metastatic head and neck cancer stratified patients with ≥2, 1, and 0 CTCs at baseline, and found a significant difference in PFS and OS between the cohorts.[10] Similar to breast cancer, ovarian cancer, and other cancers, increased CTCs seem to predict a worse prognosis, although this must be validated in SCCHN. In our cohort, we had two patients with ≥2 detectable CTCs at multiple time points, with one patient surviving for 2 months and having 170 CTCs 4 days before death.

This study found that smoking is positively associated with CTC detection on univariate analysis. Smoking has many effects on cell-to-cell adhesion, including downregulating E-cadherin, a tumor suppressor that maintains cell-cell adhesion, and promoting epithelial to mesenchyme transition (EMT)[22-29] in various cancers including prostate, lung, and oropharyngeal squamous cell carcinoma.[26,30-32] Moreover, a key step in EMT is loss of E-cadherin, leading to invasive behavior and metastasis. Tobacco smoke plays a role in this pathogenesis in SCCHN by causing hypermethylation of E-cadherin's promoter[33] and dysregulating microRNA profiles,[34] although more mechanisms have been described in lung cancer.[23] The majority of patients in this study were also HPV16 (+) which transcriptionally represses E-cadherin.[32,35] EMT promotes CTC formation,[36] thus the patients in this study had risk factors that facilitate the generation of CTCs.

In previous studies, smoking and presence of CTCs in SCCHN has not demonstrated an association: one study of 144 patients compared current smokers and nonsmokers and found no association, and another study of 48 patients did not find significance in patients with a ≥15 pack-year.[14,37] These studies used different methodologies for detecting CTCs, but in patients with lung cancer who had CTC detection by CellSearch, smoking was similarly found not to be associated with smoking.[38,39] Although these are contradictory to our findings, they suggest that the differences in detection methods and carcinoma contribute to the variability.

The lack of association with PFS and OS in this study raises the question as to whether CellSearch in SCCHN selects the most prognostic population. Recently, new technologies have allowed detection of CTCs that are not dependent on EpCAM and have shown promise.[40] With the current data available on CellSearch on patients with SCCHN, it seems that other modalities should be examined. Moreover, Nicolazzo et al describe the challenges with CellSearch and how subpopulations of CTCs with lower expression levels of EpCAM are worth investigating, especially since CTCs from patients with SCCHN detected by CellSearch do not have prognostic value.[41]

This study had limitations—our low enrollment and heterogeneity of disease weaken our conclusions. Also, treatment was not standardized within cohorts. Additionally, some patients had more sequential blood draw results than others, and several patients did not meet the endpoints of the study. Timing of blood draws in relation to treatment may have played a role in the differences in CTC counts.

Our data suggest that smoking may play a role in CTC development in patients with SCCHN. A homogenous study cohort with standardized care, increased enrollment, and regularization of blood draws during and after treatment might have allowed us to establish association between CTC and other factors more firmly, and we therefore suggest further investigation to assess efficacy and predictive value of CTCs in this patient population.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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