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Prognostic impact and potential predictive role of baseline circulating tumor cells in locally advanced head and neck squamous cell carcinoma

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ABSTRACT

Objectives: The prognostic impact of circulating tumor cells (CTCs) or circulating tumor microemboli (CTM) in locally advanced head and neck squamous cell carcinoma (LA-HNSCC) is yet to be determined, with conflicting results in previous trials. The role of induction chemotherapy (ICT) in the management of LA-HNSCC is controversial with no predictive biomarkers to guide treatment strategy in this scenario. The aim of this trial is to determine the prognostic impact of CTCs and CTM, their biomarkers expression by immunocytochemistry (ICC), and its potential role as predictors of ICT benefit in LA-HNSCC.

Materials and Methods: Prospective study, with newly diagnosed stage III/IV non-metastatic LA-HNSCC patients treated with curative intent. Blood samples analyzed for CTCs and CTM before treatment using the ISET method.

Results: A total of 83 patients were included. CTCs counts were an independent prognostic factor for overall survival (OS; HR: 1.17; 95 %CI: 1.05–1.31; p = 0.005) and progression free survival (PFS; HR:1.14; 95 %CI: 1.03–1.26; p = 0.007). Using the Lausen and Schumacher technique, 2.8 CTCs/mL for OS and 3.8 CTCs/mL for PFS were defined as the best cut-offs. CTM were detected in 27.7% of patients, correlating with worse PFS (HR = 2.70; IC95%: 1.30–5.58; p = 0.007). MRP-7 expression in CTM correlated with worse OS (HR = 3.49; 95 %CI: 1.01–12.04; p = 0.047) and PFS (HR = 3.62; 95 %CI: 1.08–12.13; p = 0.037). CTCs counts were predictive of complete response to treatment (OR = 0.74; 95 %CI: 0.58–0.95; p = 0.022) and high counts (cut-off 3.8/mL) and CTM were potential predictors of ICT benefit.

Conclusion: CTCs/CTM had significant prognostic impact and potential role as predictors of ICT benefit in LA-HNSCC.

Introduction

Head and neck squamous cell carcinoma (HNSCC) represent the seventh most common neoplasm, accounting for 700.000 new cases annually worldwide [1]. About two thirds of patients are diagnosed with locally advanced (LA) disease and are candidates to multimodality

Abbreviations: HNSCC, head and neck squamous cell carcinoma; LA, locally advanced; CTCs, circulating tumor cells; EMT, epithelial mesenchymal transition; CTM, circulating tumor microemboli; ISET, isolation by size of epithelial tumor cell; ERCC1, excision repair cross-complementation group 1; EGFR, epidermal growth factor receptor; MRP-2, multidrug resistance protein 2; MRP-7, multidrug resistance protein 7; MMP-2, matrix metalloproteinase 2; TGF-βRI, transforming growth factor beta receptor type I; ICC, immunocytochemistry; AJCC, American Joint Committee on Cancer; RT, radiotherapy; ICT, induction chemotherapy; TPF, docetaxel 75 mg/m^2 D1 + Cisplatin 75 mg/m^2 D1 + 5-FU 750 mg/m^2 D1 to D5 continuous infusion; PPF, paclitaxel 175 mg/m^2 D1 + Cisplatin 75 mg/m^2 D1 + 5-FU 500 mg/m^2 D1 to D5 continuous infusion; PCC, carboplatin AUC=2 + paclitaxel 90 mg/m^2 + Cetuximab 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly; IMRT, intensity modulated radiotherapy; OS, overall survival; PFS, progression-free survival; ROC, receiver operating characteristics; RT-PCR, reverse transcriptase-polymerase reaction.

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treatment with curative intent. However, >50% will present disease recurrence and, to date, there are no predictive biomarkers to guide the choice of primary treatment strategy [2].

Circulating Tumor Cells (CTCs) are considered pivotal in the process of metastasis and recurrence of cancer [3], invading the blood stream utilizing specific pathways as the epithelial mesenchymal transition (EMT) [4]. The ability to form clusters, the circulating tumor microemboli (CTM), allow these cells to escape from host immune attack [5].

The prognostic value of CTCs has been demonstrated in some solid cancers [6,7]. In LA-HNSCC, this role is yet to be determined, with conflicting results in previous trials, most of them utilizing cytokeratin dependent techniques [8–10]. In a previous study, with a limited cohort, we showed the potential role of CTCs/CTM in HNSCC using an isolation by size of epithelial tumor cell (ISET) technique [11].

Predictive biomarkers are desirable in cancer treatment, especially when multiple strategies are available, like LA-HNSCC [12]. Diverse pathways have been studied in HNSCC, DNA repair with excision repair cross-complementation group 1 (ERCC1) [13], epidermal growth factor receptor (EGFR) [14], β-tubulin isoforms [15], multidrug resistance proteins 2 and 7 (MRP-2 and MRP-7) [16] and EMT markers like matrix metalloproteinase 2 (MMP-2) [17] and transforming growth factor beta receptor type I (TGF-βRI) [18]. In most studies, the biomarkers expression profile was accessed at baseline, in pretreatment tissue biopsy specimen. However, treatments such as radiotherapy (RT) can alter tumor microenvironment [19], thus CTCs evaluation could better reflect the tumor clonality during treatment.

Our aim was to determine the detection rate of CTCs in LA-HNSCC using ISET, the prognostic impact and potential predictive role of CTCs counts, the presence of CTM and expression of biomarkers in these cells by immunocytochemistry (ICC), in patients treated with a curative intent with different multimodality strategies.

Methods

Study design and patient population

Prospective, single-center, non-randomized study, approved by the Medical Ethical Committee of AC Camargo Cancer Center, São Paulo, Brazil (1777/13). Patients with LA-HNSCC newly diagnosed with non-metastatic disease (stages III, IVA and IVB by AJCC 7th edition) were invited and signed the Informed Consent Term. Inclusion criteria: ≥ 18 years; histological diagnosis of squamous cell carcinoma; primary site in the oral cavity, oropharynx, larynx, hypopharynx or cervical disease with occult primary site; treatment with curative intent. Exclusion criteria: surgery or surgical procedure in the last three days or previous history of another active neoplasm in the last 5 years.

Treatment

Patients were candidates to one out of 4 treatment strategies, according to the physician discretion: surgery followed by RT combined with cisplatin-based chemotherapy (adjuvant chemoradiation), upfront RT combined with cisplatin-based chemotherapy (definitive chemoradiation) or Cetuximab (definitive bioradiation) and induction chemotherapy (ICT) followed by chemoradiation or bioradiation. Cisplatin-based chemoradiation consisted of weekly cisplatin 40 mg/m^2 for 6 to 7 cycles or 100 mg/m^2 every 3 weeks for 3 cycles concurrent with RT. Bioradiation consisted of Cetuximab 400 mg/m^2 loading dose 1 week before, followed by 250 mg/m^2 weekly concurrent with RT. ICT protocols allowed were TPF (docetaxel 75 mg/m^2 D1 + cisplatin 75 mg/m^2 D1 + 5-FU 750 mg/m^2 D1 to D5 continuous infusion every 3 weeks), PPF (paclitaxel 175 mg/m^2 D1 + cisplatin 75 mg/m^2 D1 + 5-FU 500 mg/m^2 D1 to D5 continuous infusion every 3 weeks) both for 3 cycles or PCC (carboplatin AUC = 2 + paclitaxel 90 mg/m^2 + Cetuximab 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly) for 6 weeks. Treatment decision was based on clinical features and most of the cases were discussed on multidisciplinary tumor board meetings. Physicians were blind to the CTCs results.

All RT planning was computed tomography-based with either 3D conformal RT or, preferably, intensity modulated RT (IMRT) with sliding window technique. The prescribed dose was 70 Gy to macroscopic lesions, either the primary tumor or involved lymph nodes, 60 Gy in the primary tumor bed or high-risk uninvolved lymph nodes and 54 Gy in low-risk uninvolved lymph-nodes in 33 to 35 daily fractions, 5 fractions per week, in combination or not with chemotherapy or Cetuximab.

Circulating tumor cells analysis

Whole peripheral blood samples (10 mL) were collected in EDTA tubes before any treatment. For CTCs characterization and counting, ISET (Isolation by SizE of Tumor Cells, RareCells Diagnostics, Paris, France) was used. Samples were diluted with ISET Buffer^{TM} 1:10 and after incubation (10 min), were filtered, washed (PBS) and stored at −20 °C after dried. For ICC, membrane spots were cut and placed into a 24-well plate. For antigenic recovery, each spot was incubated with Target Retrieval Solution (1x) (Dako^{TM}) and heated in a microwave water. Then, plate was maintained at room temperature (20 min). Cells were permeabilized with 0.2% TBS Triton X-100 (5 min, room temperature). After wash, membranes were incubated (15 min, in the dark, room temperature) with 3% hydrogen peroxide solution and washed. The chosen antibody (Table S1 of the supplementary appendix) was applied (2 h). Double immunocytochemical labeling protocol was performed. So, after incubation with primary antibody, spots were washed, incubated in Dual Long System HRP (Dako^{TM}) and revealed by the chromogen DAB (Dako^{TM}). Next, we performed incubation with the second antibody and another incubation with the Rabbit/Mouse (LINK) (Envision^{TM} G/2 System/AP Dako^{TM}). After washing, spots were incubated with AP Enzyme (Enhancer/Dako^{TM}). The second antibody was revealed by Permanent Red. For reading, spots were stained with hematoxylin and examined in light microscope, BX61-Olympus coupled to a high-resolution digital camera SC100-Olympus (Tokyo, Japan). CTCs were characterized according to the following cytopathological criteria [20]: nuclear size ≥ 16 μm, irregularity of the nuclear contour, presence of visible cytoplasm, nucleus-cytoplasm ratio > 0.8. To those, we added the negativity for CD45 expression (leukocyte marker). When missing any of the described criteria, cells were classified as atypical (not counted). Results were given in number of CTCs/mL, counting CTCs in ≥ 4 spots of the membrane [20] and description of CTM presence, defined as clusters of ≥ 3 CTCs.

For positive controls, we used the lineages known to express each antibody spiked in healthy blood and for negative controls, spiked lineages that, accordingly to Protein Atlas, do not or weakly express each antibody (https://www.proteinatlas.org/ accessed on September 13th 2013) [21]. All lineages were maintained in culture and filtered on ISET and then subjected to ICC. We also tested blood from 5 healthy individuals, to show the ISET specificity, although we have shown the ISET specificity and discussed about it previously [22] (Figure S1 in the supplementary appendix).

Statistical analysis

Patient characteristics are expressed as absolute (n) and relative (%) frequencies for qualitative variables and as the mean, median, minimum, maximum and standard deviation (SD) for quantitative variables. To assess a possible association between two qualitative variables the chi-square test or Fisher's exact test were used, as appropriate.

For the comparisons between independent groups, Student's parametric *t* test or the Mann-Whitney U non-parametric test (two independent groups) or the Kruskal-Wallis (≥3 independent groups) were used, followed by a Bonferroni Post Hoc test when adequate.

Overall survival (OS) and progression-free survival (PFS) analyses

were estimated using the Kaplan-Meier estimator (comparison between curves by the log-rank test). In order to determine a cut-off point for the CTCs count in relation to OS and PFS, the technique proposed by Lausen and Schumacher [23] was applied. It aims to obtain the "best" cut-off point value to "discriminate" the survival curves. In each analysis, the maximally selected log-rank for cut-off points between 5% and 95% of continuous measure was considered. In addition, Cox semiparametric proportional hazards model was fitted to describe the relationship between the independent variables and the time until death/recurrence. All independent variables with a p-value of<0.10 in the simple Cox model were considered in the multiple Cox model, and other variables could be included according to clinical relevance. The variables of the final multiple Cox regression model were selected using the stepwise (backward) method with an input p of 0.10, thus obtaining the final model. In all models, the assumption of proportionality was evaluated using scaled Schoenfeld residuals and global test. In all cases, we have evidence that the effects of covariates are constant over time, thus justifying the use of the Cox model. OS was calculated from the date of diagnosis until death; PFS from the date of diagnosis until disease progression/recurrence. Response to treatment was assessed according to the RECIST 1.1 criteria [24] by both the assistant physician/radiologist.

A cut-off point for the CTCs counts and the complete response to treatment was obtained from the Receiver Operating Characteristics (ROC) curve, in which the point was chosen based on an optimization of Youden statistic.

The significance level was fixed at 5% (p-value <0.05 were considered significant). Data analysis was performed using R software version 3.5 (R Core Team, 2018).

Results

Here we describe the results of the final analysis of all patients included in this trial. The preliminary analysis of the first patients was published in 2017 [11]. Patients (n = 83) were included between January 2014 to November 2017, the majority were male (83%), median age of 60 years (25–83), with primary site in oropharynx (50.6%) and very locally advanced disease (T3/T4 in 70% and ≥ N2b in 67.5% according to AJCC 7th Edition). Non-surgical strategy was done in 80% of the patients (n = 67), half of them (n = 33) with ICT and the other half with upfront RT, concurrent with cisplatin (n = 19) or Cetuximab (n = 15). Surgery was done in 20% (n = 16) followed by adjuvant chemoradiation (Table 1).

The detection rate of CTCs in the entire population was 94% (78/83 patients), with a median of 3.5 CTCs/mL (SD: 3.2; variation: 0–19), and no difference between the adjuvant (93.7%) and definitive (94%) groups. There was no correlation between CTCs counts and clinical characteristics, except for N stage, with significantly higher CTCs counts for N2/N3 disease versus N0/N1 (p = 0.024).

With a median follow-up of 27.6 months, baseline CTCs counts were an independent prognostic factor for both OS and PFS. For each increase of 1 CTC/mL, there was a 17% increase in the risk of death (HR: 1.17; 95 %CI: 1.05–1.31; p = 0.005) and a 14% increase in the risk of progression (HR:1.14; 95 %CI: 1.03–1.26; p = 0.007). The models of the multivariate analysis for OS and PFS accessing the prognostic impact of CTCs as a continuous variable are shown in Tables S2 and S3 of the supplementary appendix. The variables described in the multivariate analysis were then selected using the stepwise (backward) method, thus obtaining the final values of HR, confidence intervals and p value described here.

Using the Lausen and Schumacher technique [23], to establish cut-off points in the CTCs counts that could discriminate survival curves, we identified 2.8 CTCs/mL for OS and 3.8 CTCs/mL for PFS as the best cut-offs values. Patients with ≤ 2.8 CTCs/mL had a significantly better OS, with 2-years (2y) OS rate of 88% and median OS not reached versus 2y OS of 70.3% and median of 52 months for CTCs higher than 2.8/mL (HR = 0.30; 95 %CI: 0.11–0.83; p = 0.015) (Fig. 1). For PFS, patients with CTCs ≤ 3.8/mL had a significantly better PFS, with 2y PFS rate of

Table 1
Demographic characteristics of the study population.

Characteristic	n	%
Age		
Median	60	–
Variation	25–83	-
Gender		
Male	69	83
Female	14	17
Primary Site		
Oropharynx	42	50,6
Oral Cavity	14	16,9
Larynx	13	15,7
Hypopharynx	10	12
Occult Primary	4	4,8
p16 (oropharynx only)	42	
Positive	22	52,3
Negative	3	7,1
Unknown	17	40,4
Smoking		
< 10 pack/years	25	30,1
≥ 10 pack/years	58	69,9
T Stage		
T0	8	9,6
T1	4	4,8
T2	13	15,7
T3	25	30,1
T4	33	39,7
N Stage		
N0	15	18,1
N1	8	9,6
N2a	4	4,8
N2b	20	24,1
N2c	19	22,9
N3	17	20,5
AJCC Stage		
III	16	19,3
IVA	46	55,4
IVB	21	25,3
Definitive non-surgical treatment	67	80,7
Induction Chemotherapy	33	49,2
Upfront Radiotherapy + Cisplatin	19	28,3
Upfront Radiotherapy + Cetuximab	15	22,3
Upfront surgery followed by adjuvant treatment	16	19,3
Adjuvant Radiotherapy + Cisplatin	16	100

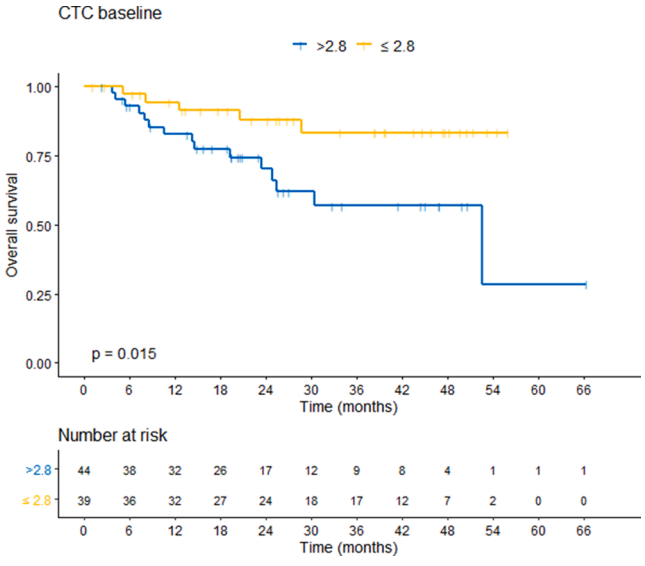

Fig. 1. Kaplan Meier curves for overall survival according to baseline CTCs counts, with a cut-off point of 2.8/mL.

71.8% and median PFS not reached versus 37% and 17.4 months respectively, for patients with CTCs higher than 3.8/mL (HR = 0.32; 95 %CI: 0.15–0.67; p = 0.001) (Fig. 2). As an exploratory analysis, only 5 out of 83 patients had no CTCs detectable. Of these, one died from complications of the treatment and the other 4 were alive without evidence of disease at 1+, 17+, 22 + and 40 + months.

CTM were detected in 27.7% of patients (n = 23) with a significant correlation with higher CTCs counts (p < 0.001). CTM was significantly correlated with worse PFS, with a median PFS and 2y PFS of 16.7 months and 43% for CTM positive versus median not reached and 65.3% respectively for patients without CTM (HR = 2.70; IC95%: 1.30–5.58; p = 0.007) (Fig. 3). For OS, although a numeric difference is seen, with 2y OS rate of 62.8% versus 84.3% for presence and absence of CTM respectively, this result was not statistically significant (HR = 2.32; IC95%: 0.95–5.66; p = 0.064) (Fig. 4).

EGFR, ERCC1, β-tubulin III, MRP-2, MRP-7, TGFβRI and MMP2 expressions were analyzed in the CTCs and there was no significant correlation neither with OS nor PFS (Table S4 of supplementary appendix). For CTM, we found a significant correlation of MRP-7 expression with worse OS (HR = 3.49; 95 %CI: 1.01–12.04; p = 0.047) and PFS (HR = 3.62; 95 %CI: 1.08–12.13; p = 0.037) (Figures S2 and S3 of supplementary appendix), β-tubulin III expression and worse OS (HR = 4.74; 95 %CI: 1.02–21.94; p = 0.046) and EGFR expression and worse PFS (HR = 2.88; 95 %CI: 1.00–8.30; p = 0.05) (Table S5 of supplementary appendix).

To analyze the potential predictive impact of baseline CTCs counts and CTM, only patients treated with a non-surgical approach (n = 67) were evaluated. In this population, response according to RECIST is a possible endpoint, and an analysis according to treatment strategy comparing ICT versus upfront RT is of interest. In this subgroup of patients, baseline CTCs was also an independent prognostic factor for both OS (HR = 1.23; 95 %CI: 1.09–1.39; p < 0.001) and PFS (HR = 1.22; 95 % CI: 1.08–1.37; p = 0.001) and the cut offs of 2.8 CTCs/mL (HR = 0.33; 95 %CI: 0.10–1.05; p = 0.051) for OS and 3.8 CTCs/mL for PFS (HR = 0.28; 95 %CI: 0.11–0.67; p = 0.004) were good prognosticators.

To correlate the baseline CTCs counts and response to treatment, patients that underwent non-surgical definitive treatment and had response evaluated (n = 61/67) were analyzed. Reasons for not having response evaluation were lost of follow up (n = 3) and RT interruption for complications (n = 3). CTCs counts were significant correlated with response to treatment, for each increase of 1 CTC/mL there was a decrease of 26% in the odds of complete response to treatment (OR = 0.74; 95 %CI: 0.58–0.95; p = 0.022). To try to establish a cut-off point that predicted complete response, a ROC curve was constructed with an AUC = 0.675 (95 %CI: 0.511–0.837) and an optimal cut-off point of 3.1 CTCs/mL that, however, was not statistically significant (OR = 0.48; 95 %CI: 0.15–1.15; p = 0.212).

Evaluating the predictive role of CTCs counts and presence of CTM according to treatment strategy, patients treated with non-surgical approach were divided in two groups: upfront RT (n = 34) or ICT followed by RT (n = 33). Here, patients that received ICT were younger (mean age of 56.2 versus 64.8 years; p = 0.001), had higher smoking load in pack years (p < 0.001), had more advanced N stage (p < 0.001) and AJCC stage (p < 0.001) (Table S6 of supplementary appendix). Despite this, there were no difference in OS (HR = 0.50; 95 %CI: 0.17–1.47; p = 0.204) or PFS (HR = 1.21; 95 %CI: 0.52–2.80; p = 0.655) between the two groups. Testing the cut-offs provided by Lausen and Schumacher technique we found that 3.8 CTCs/mL provided a numeric difference of OS in relation to treatment strategy. Patients with CTCs ≤ 3.8/mL had a 2-y OS of 88.2% when treated with induction CT versus

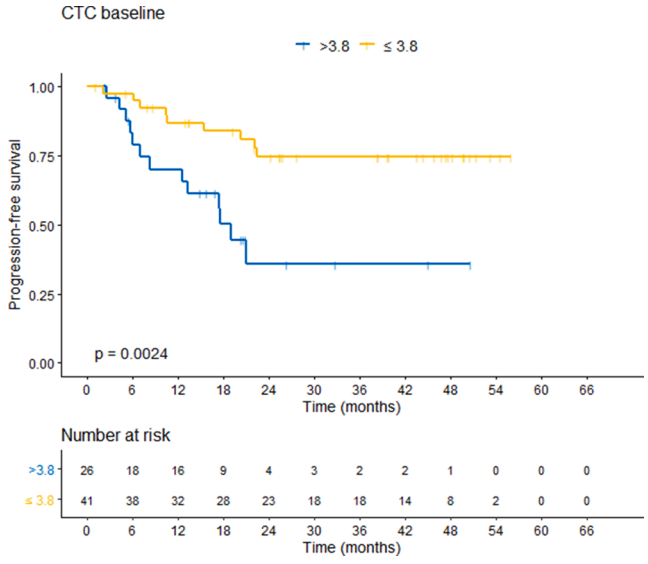

Fig. 2. Kaplan Meier curves for progression free survival according to baseline CTCs counts, with a cut-off point of 3.8/mL.

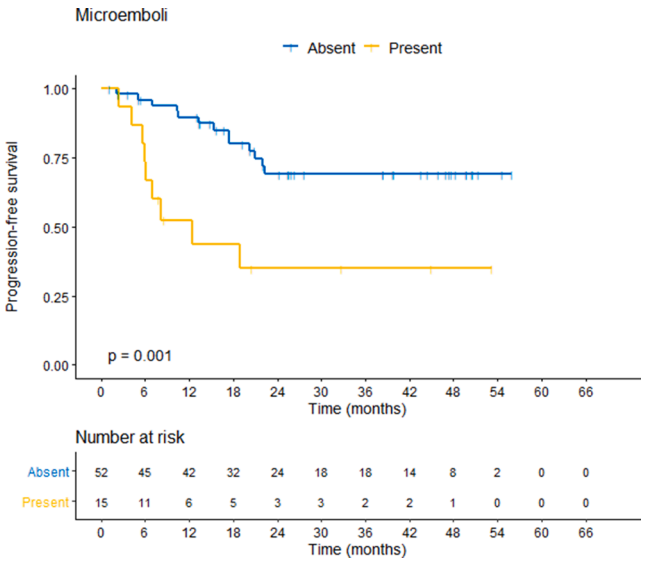

Fig. 3. Kaplan Meier curves for progression free survival according to presence or not of circulating tumor microemboli (CTM).

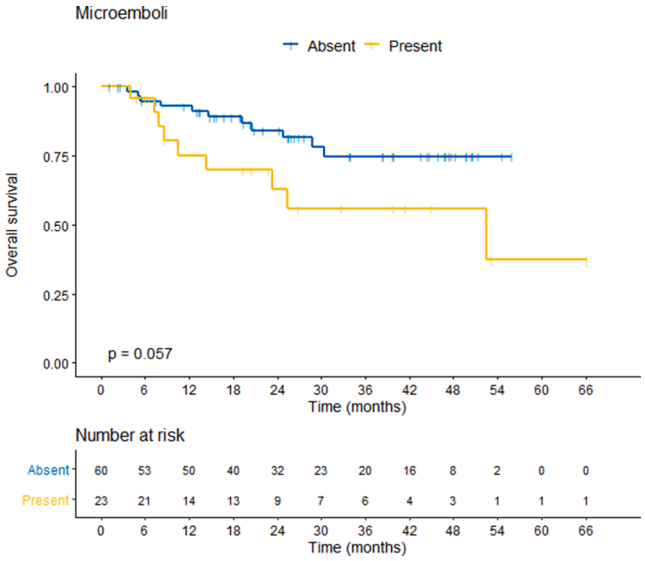

Fig. 4. Kaplan Meier curves for overall survival according to presence or not of circulating tumor microemboli (CTM).

79.8% for upfront RT (HR = 0.55; 95 %CI: 0.10–0.84; p = 0.470), while patients with CTC > 3.8/mL had a 2y OS of 74.8% for induction CT versus 59.8% for upfront RT (HR = 0.32; 95 %CI: 0.07–1.38; p = 0.112) (Figs. 5A and B).

The same was observed when analyzing the presence of CTM. Patients with CTM treated with upfront RT had a significantly worse OS in comparison to those without CTM treated with induction CT (HR = 0.13; 95 %CI: 0.02–0.59; p = 0.009) or upfront RT (HR = 0.22; 95 %CI: 0.06–0.84; p = 0.027), and non-significant in comparison to patients with CTM receiving induction CT (HR = 0.29; 95 %CI: 0.05–1.64; p = 0.164) (Fig. 6).

Discussion

We showed that, with ISET method, the detection rate of CTCs in LA-HNSCC is extremely high, above 90%, even in a population with no known metastatic disease, including patients previous surgically treated. These numbers compare favorably with other CTC isolation techniques, especially those dependent of cytokeratin staining, such as

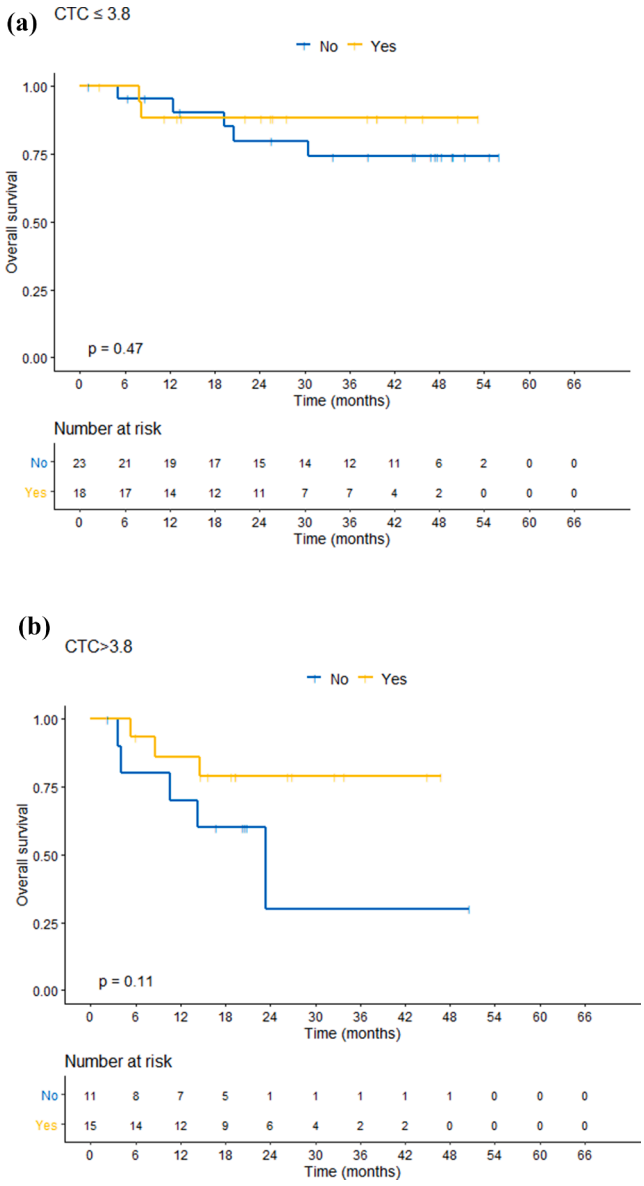

Fig. 5. Kaplan Meier curves for overall survival according to the use of induction chemotherapy or not, in patients with CTCs ≤ 3.8/mL (5A) and > 3.8/mL (5B).

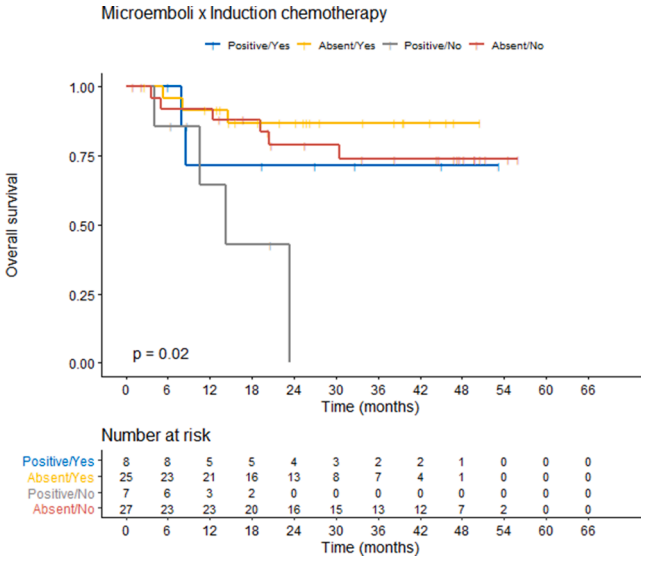

Fig. 6. Kaplan Meier curves for overall survival according to the use of induction chemotherapy or not and the presence of circulating tumor microemboli (CTM).

CellSearch (Menarini Biosystems), ranging from 12.5% to 40% in locally advanced disease [25–29] and 21% to 41% in metastatic disease [30,31] or RT-PCR based techniques, ranging from 6.5% to 63% in LA-HNSCC [32–38]. Studies that utilize microfiltration assays, that include a size dependent step, present higher sensitivity in indirect comparison (47.8 to 90.6%) [39,40], and also in a direct comparison [41] and this is also observed with ISET in other tumor types [42–44]. These differences are probably due to two factors: the literature has shown that CTCs from patients with head and neck cancer express low amount of cytokeratin, a common marker used by many different methods based in antibody separation [45] and, methods based in physical properties, independent of antibody selection, can isolate CTCs that are under mesenchymal phenotype, not only those with epithelial markers. We and others have shown that CTCs from head and neck cancer can express mesenchymal markers [11,30,45,46]. Here, CTCs counts were correlated with N stage, but not with other characteristics, which could indicate a relation between higher CTCs counts and micrometastatic disease, since advanced N stage is directly correlated with higher risk of distant recurrence [47].

Baseline CTCs counts had a significant correlation with survival. To our knowledge this is one of the first trials to demonstrate an independent prognostic impact of quantitative analysis of CTCs both on OS and PFS. For example, all trials using the CellSearch [25–29] analyzed CTCs qualitatively, and only one showed a significant correlation with PFS [28], and none out of three metanalysis could demonstrate an impact on OS [8–10]. We could identify cut-off points for OS and PFS and, in addition, using CTCs as a continuous variable, we demonstrate that the higher the CTCs counts, the worse the prognosis. CTM was significantly correlated with worse PFS. There is evidence that the formation of CTCs clusters confers an advantage in the metastasis process [48,49], with data showing absence of proliferation or apoptosis markers in the CTM [50,51], which could confer chemotherapy resistance [52,53], and also a potential of enhanced immune evasion [54]. There is data in other tumor types correlating CTM with worse outcomes [44,55–58], but not in head and neck cancer.

Our findings highlight the importance of CTCs counts/CTM presence as prognostic biomarkers in LA-HNSCC, and the possibility of incorporating these in staging and the decision-making process of treatment strategy. Based on this rational we analyzed the predictive potential of these biomarkers. Baseline CTCs counts were a predictor of complete response to treatment, with higher counts significantly related with a lower chance of response, but a cut-off point could not be determined.

Dividing the non-surgical patients into two subgroups, the ones receiving ICT followed by (chemo)radiation and the ones treated with upfront (chemo/bio) radiation, patients with lower CTCs counts (below 3.8/mL) and no CTM appeared to have no survival advantage of receiving induction, whereas patients with higher CTCs counts or presence of CTM that received ICT had non-significant advantage in OS compared to upfront radiation. These analyses have limitations such as the non-randomized nature of this trial and the non-significant difference in OS. However, it suggests that baseline CTCs counts and presence of CTM could be predictors of ICT benefit and should be further explored in larger prospective clinical studies. Moreover, trials comparing upfront chemoradiation versus ICT followed by (chemo)radiation have controversial results [59–63] and even those that select patients based on the risk of distant recurrence and clinical characteristics had negative results [62,63], which demonstrates the need of better predictive biomarkers of ICT benefit.

Other recognized limitations of our trial are the small cohort of patients representing different anatomical subsites with inherent differences in prognosis and the absence of validation in an independent cohort. However, it represents the real world of LA-HNSCC, since all these anatomical subsites have multiple treatment strategies that can be employed, like upfront surgery or non-surgical approaches, with the non-surgical treatment comprising the use of induction chemotherapy or not, and more than one option of systemic therapies as radiosensitizers, with no predictive biomarkers identified to guide the choice of these multiple strategies. Our aim was exactly to try to identify predictive biomarkers inside this heterogeneous population leading to better selection and a more personalized treatment approach. In this context we choose to include a more representative population of LA-HNSCC to generate hypothesis that could be further explored in future trials with higher number of patients and more controlled clinical scenarios.

In summary, CTCs and CTM detected by ISET are feasible, have a significant prognostic impact and a potential role as predictors of ICT benefit in LA-HNSCC.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2021.105480.

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