Original Article

Monitoring with circulating tumor cells in the perioperative setting of patients with surgically treated stages I–IIIA NSCLC

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Background: Surgery is regarded as the treatment's cornerstone for early stage and locally advanced non-small cell lung cancer (NSCLC) whenever the tumor is considered resectable. Liquid biopsy is one of the most promising research areas in oncology in the last 10 years, providing a useful non-invasive tool to detect and monitor cancer. The prognostic value of circulating tumor cells (CTCs) has been studied in different cancer types and had been related with a higher risk of relapse and worse prognosis. The aim of this study is to evaluate the prognostic value of CTC detection in patients with stage I–IIIA NSCLC treated with surgery.

Methods: We conducted a prospective, single-center study of 180 consecutive patients with resected and pathological confirmed stage I to IIIA (TNM AJCC/UICC 8th edition) NSCLC. Patients' blood samples were processed and CTCs were characterized before and after the surgery. A cohort of patients had CTC determination after chemotherapy and surgery. Cut-off points were established in 1 and 5 CTCs for statistical analysis.

Results: A proportion of 76.7% had at least 1 CTC before the surgery, and 30.6% had 5 or more, while 55.9% had at least 1 CTC after surgery, and 8.3% had 5 or more. We found no correlation between preoperative CTC detection for a cut-off of 5 with neither overall survival (OS) [hazard ratio (HR): 0.99, P=0.887], disease-free survival (DFS) (HR: 0.95, P=0.39) nor relapse (32.7% *vs.* 28.8%, P=0.596). We also did not find a correlation between postoperative CTCs detection for a cut-off of 5 with either OS (HR: 1.01, P=0.808), DFS (HR: 0.95, P=0.952) or relapse (26.7% *vs.* 29.5%, P=0.83). The mean change in the number of CTCs over time between preoperative and postoperative samples was 2.13, with a standard deviation of 6.78.

Conclusions: Despite the large cohort of patients included in this study, CTC monitoring in the perioperative setting was not correlated with relapse, DFS or OS in our study, and therefore cannot be recommended as a reliable biomarker for minimal residual disease (MRD) after surgery.

Keywords: Non-small cell lung cancer (NSCLC); circulating tumor cell (CTC); liquid biopsy; surgery

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Introduction

Lung cancer is the main cause of cancer-related death and the second most important tumor in terms of incidence in both sexes, with an estimated 2.2 million new cases and 1.8 million deaths worldwide. Prognosis depends mainly on the tumor stage at diagnosis, but it is nevertheless estimated that fewer than 26% of non-small cell lung cancer (NSCLC) patients will be alive 5 years after being diagnosed (1).

Surgery is regarded as the cornerstone of the treatment for early and locally advanced NSCLC whenever the tumor is considered resectable. According to the European guidelines, neoadjuvant systemic therapy should be offered to patients with resected stage II or III NSCLC and resected stage IB with a tumor >4 cm (2). Recent studies support the use of neoadjuvant chemoimmunotherapy in these circumstances, and have reported promising results in terms of survival (3,4).

Despite optimal treatment, 5-year survival rates remain lower than expected in early NSCLC compared with other cancer types, probably because of the risk of relapse after surgery, which is nearly 25% for local progression and an additional 13% for disrtal relapse in stages I and II (5).

Liquid biopsy has been one of the most promising research areas in oncology in the last 10 years, providing a useful non-invasive tool with which to detect and monitor cancer. The main biomarkers analyzed by liquid biopsy are circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), with others, including RNA, tumor-educated platelets, and extracellular vesicles also being important (6).

CTCs are intact and frequently viable cells that can be discriminated from normal immune cells in blood by using antibodies to epithelial and/or mesenchymal proteins (e.g., EpCAM, cytokeratins, vimentin, or N-cadherin), by negative selection through leukocyte depletion using anti-CD45 antibodies, or based on physical properties such as size, deformability, density, and electrical charge (7).

ctDNA consists of small fragments of free nucleic acid harboring specific tumor mutations that can be detected by conventional methods, such as polymerase chain reaction (PCR), or new molecular models like next generation sequence (NGS) (8).

The prognostic value of CTCs has been studied in different cancer types, such as colorectal cancer, in which they have proved to be associated with a higher risk of relapse and worse prognosis (9). However, in lung cancer, and particularly in its early stages, despite some retrospective studies indicating such a relationship, there is a lack of prospective information to determine whether CTC counts are correlated with prognosis (10-13). In this context, the aim of the study is to evaluate the prognostic value of CTC detection in patients with stage I–IIIA NSCLC treated with surgery. We present this article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-827/rc).

Highlight box

Key findings

- Circulating tumor cell (CTC) monitoring in the perioperative setting of resectable lung cancer does not correlate with relapse or survival.

What is known and what is new?

- There is evidence that support the prognostic role of CTC in different cancer types but it is not clear if this can be translated to early-stage lung cancer.
- To our knowledge, this study has one of the largest sample sizes and our results show that CTC monitoring cannot be recommended as a reliable biomarker in this setting.

What is the implication, and what should change now?

- There is an unmet need to develop novel biomarkers that can be used in daily clinical practice and help oncologists to assess prognosis patients with early stage lung cancer. Given the fact that CTC monitoring does not seem to be a reliable biomarker, we should probably focus on other biomarkers such as circulating tumor DNA.

Methods

Study design

We performed a prospective, single-center study of 180 consecutive patients with resected and pathological confirmed stage I to IIIA (TNM AJCC/UICC 8th edition) NSCLC. An initial sample was collected from radial venous blood between tumor diagnosis and surgery between 2013 and 2018. A second sample was collected between 7 days and 6 months after surgery. In 56 patients, we collected a third blood sample after they had completed adjuvant chemotherapy (*Figure 1*). All patients were followed up to monitor their survival.

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The Hospital Universitario Puerta de Hierro Ethics Committee (No. 296) approved the study. Written informed consent was obtained from every patient prior to their participation. Samples were processed and analyzed by the Flow Cytometry Core Facility

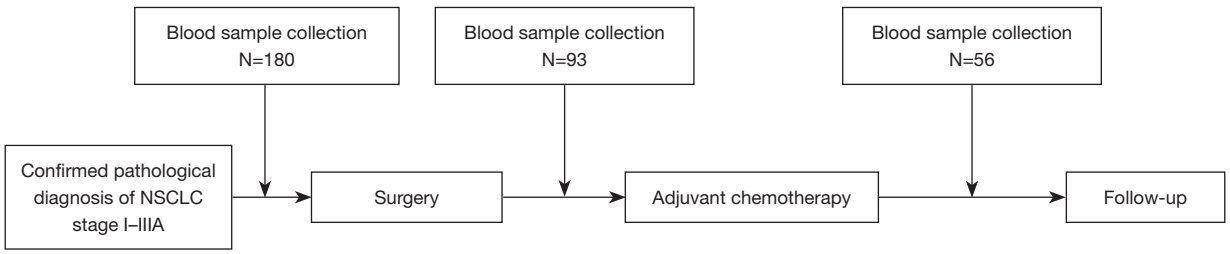

Figure 1 Timeline of the blood sample collection during the study period. NSCLC, non-small cell lung cancer.

and Confocal Microscopy Core Facility of the Biomedical Sciences Research Institute Puerta de Hierro-Segovia de Arana (IDIPHISA) (Majadahonda, Madrid, Spain).

The aim of the study is to evaluate the prognostic value of CTC detection in patients with stage I–IIIA NSCLC treated with surgery.

Enrichment and detection of circulating tumor cells

Patients' blood samples were processed according to the method of isolation and characterization of CTCs that has been established by our group and which has been reported elsewhere (14,15).

Briefly, for CTC analysis, blood was collected in CellSave Preservative Tubes (Veridex). Pre-enrichment of CTCs was performed by the double-density gradient method (HISTOPAQUE-1077/HISTOPAQUE-1119). CTCs were enriched using selective positive immunomagnetic cell separation, with EpCAM microbeads. The magnetically labeled cell suspension was then purified and enriched in a magnetic field using an AutoMACS (Miltenyi Biotec) magnetic separator. After capture, reagents were added for intracellular and extracellular phenotypic identification of CTCs by flow cytometry and confocal microscopy. The enriched fraction was fluorescently labeled with anti-human CD45-APC, anti-human CD326-EpCAM PE, a nuclear dye to detect viable cells, and anti-cytokeratin-FITC. Intracellular staining was performed by fixing in methanol and washing in PBS. Samples were incubated for 1 hour at room temperature, mounted in PBS/glycerol, and quantified by confocal microscopy (*Figure 2*).

Samples were analyzed by flow cytometry using a MACSQuant flow cytometer (Miltenyi-Biotec) equipped with three solid-state lasers, which allowed simultaneous measurement of up to 10 parameters. Microscopy images were collected with a TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany) equipped with 20× 0.4 lens and 3× optical zoom. Data were analyzed using sensitive MACS Quantify™ Software v2.5 (Miltenyi Biotec) and Leica LASFlite (Leica Microsystems, Wetzlar, Germany).

We defined cut-offs for CTC detection of 1 and 5: patients with CTC <5 were considered to have low levels, and those with CTC ≥5 were considered to have high levels, as has been previously reported in the literature (16-22). Comparisons of these two patient groups were then made.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v.26. Overall survival (OS) was defined as the period between the time of surgery and date of death from any cause. Disease-free survival (DFS) was defined as the period from the time of surgery to the date of confirmed relapse of the disease or death from any cause, whichever occurred first.

For group comparisons, we used ANOVA, Student's *t*-tests, Chi Square contingency tests and Pearson's product correlation. Kaplan-Meier analyses and the log-rank test were used for survival analyses. Values of $P<0.05$ were considered significant.

To interpret the variation between preoperative and postoperative CTCs we subtracted the postoperative from the preoperative value. Patients with a value of ±10 were considered to have significant variation.

Relapse was classified as oligometastatic or multiple. An oligometastatic relapse was concluded when it was limited to five metastases in no more than three organs, including lung metastases.

We stratified the results by stages IA to IIB, and IIIA.

Results

Patient characteristics

Of the 180 patients, 57 were women (31.7%) and 123 were men (68.3%), with a median age at diagnosis of 66 years.

In relation to tumor histology, 55% presented

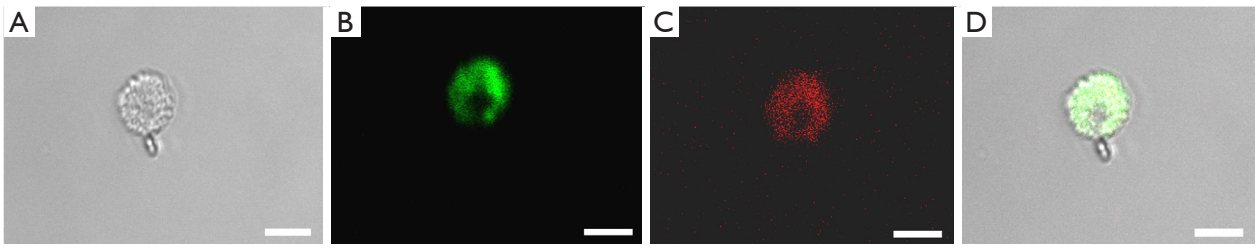

Figure 2 CTC image. (A) Phase contrast; (B) labelling with cytokeratin (green); (C) labelling with EpCAM (red); (D) merge. Bars correspond to 20 µm. CTC, circulating tumor cell.

adenocarcinoma, 35% squamous, and 10% other pathologies. The stage distribution was 28.9% IA, 24.4% IB, 11.7% IIA, 10.6% IIB, and 24.4% IIIA. Full results regarding clinical characteristics and CTC distribution are shown in *Table 1*.

CTC distribution

Complete data are shown in *Table 2*. Taking 1 CTC as the cut-off, CTCs were detectable in 76.7% of patients before performing surgery, in 55.9% once surgery had been performed, and in 66.1% once chemotherapy and surgery had been completed.

Considering 5 CTCs as the cut-off, CTCs were detectable in 30.6% of patients before performing surgery, in 8.3% once surgery had been performed, and in 23.2% once chemotherapy and surgery had been completed.

Tables 3,*4* show the distribution of clinical characteristics with respect to patients having detectable CTCs before surgery, and for a cut-off value of 5, respectively.

Relationship between preoperative CTCs and clinical parameters

We found no significant correlation between the two groups related to age (P=0.97), sex (P=0.32), SUVmax (P=0.73), pathological nodal involvement (P=0.74), pathological stage (P=0.85), or pattern of relapse (P=0.11). A higher proportion of patients with high levels of CTC were found in squamous cell carcinoma than in adenocarcinoma cases (P=0.02).

There were no differences in the proportions relapsing between the patients who had at least one presurgical CTC and those with no presurgical CTCs (31.9% *vs.* 23.8%, P=0.487).

Comparing patients who had at least five and those with fewer than five presurgical CTCs revealed no differences in the proportions relapsing (32.7% *vs.* 28.8%, P=0.596), the OS, with an HR of 0.99 (0.90–1.01, P=0.887), or DFS, with a HR of 0.95 (0.86–1.06, P=0.39). OS is shown in *Figure 3*.

For stage I–II, we found no significant correlation between the two groups related to age (P=0.33), sex (P=0.79), SUVmax (P=0.61), pathological nodal involvement (P=0.19), histology (P=0.16), pathological stage (P=0.66), or pattern of relapse (P=0.28). There were no differences between the two study groups (considering a cut-off of 5 CTCs) with respect to OS, with an HR of 0.97 (0.83–1.15, P=0.80), or to DFS, which had an HR of 0.95 (0.80–1.13, P=0.55).

In stage IIIA, we found no significant correlation between the groups related to age (P=0.47), sex (P=0.35), SUVmax (P=0.74), pathological nodal involvement (P=0.07), or pattern of relapse (P=0.33). The distribution of CTCs differed between stage IIIA adenocarcinomas and squamous cell carcinomas (P=0.02). There were no differences between the two study groups (considering a cut-off of 5 CTCs) for OS, with an HR of 0.99 (0.88–1.10, P=0.84), or in DFS, which had an HR of 0.94 (0.83–1.05, P=0.29).

Relationship between postoperative CTCs and clinical parameters

Samples collected during the prespecified period after surgery were collected from 93 patients.

We found no significant correlations between the two groups related to age (P=0.60), sex (P=0.62), SUVmax (P=0.74), histology (P=0.54), pathological nodal involvement (P=0.20), pathological stage (P=0.37), or pattern of relapse (P=0.33).

There were no differences in the proportions of relapsing cases between patients who had at least one and those with no postsurgical CTCs (30.8% *vs.* 26.8%, P=0.678).

We found no differences between the patients who had five or more postsurgical CTCs and patients who had fewer than five postsurgical CTCs with respect to relapse (26.7% *vs.* 29.5%, P=0.83), OS, with an HR of 1.01 (0.91–1.12, P=0.808), or DFS, which had an HR of 0.95 (0.91–1.10, P=0.952). OS is shown in *Figure 4*.

For stage I–II, we found no significant correlations

Table 1 Patient characteristics and correlation with CTC isolation

Clinical characteristics	Distribution
Sex	
Male	123 (68.3%)
Female	57 (31.7%)
Age at diagnosis, years, mean (95% CI)	66.2 (64.9–67.5)
Histology	
Adenocarcinoma	99 (55.0%)
Squamous cell carcinoma	63 (35.0%)
Other histologies	18 (10.0%)
PD-L1 status	
Unknown	157 (87.2%)
<1%	14 (1.8%)
1–49%	3 (3.6%)
≥50%	6 (7.2%)
Stage	
IA	52 (28.9%)
IB	44 (24.4%)
IIA	21 (11.7%)
IIB	19 (10.6%)
IIIA	44 (24.4%)
Pathological nodal involvement, mean (95% CI)	0.7 (0.4–0.9)
SUVmax at diagnosis, mean (95% CI)	9.1 (8.2–10.1)
Chemotherapy	
Adjuvant	40 (22.2%)
Neoadjuvant	29 (16.1%)
Radiotherapy	
Adjuvant	26 (14.4%)
Relapse	
Metastatic	30 (55.6%)
Local	24 (44.4%)
Type of metastatic relapse	
Oligometastatic	17 (56.7%)
Multiple	13 (43.3%)
Follow-up, months, mean (95% CI)	45.8 (41.4–50.2)

CTCs, circulating tumor cells; SUVmax, maximum standardized uptake value.

Table 2 CTC distribution

CTCs collecting time	CTCs distribution
CTCs before surgery (n=180)	
<1	42 (23.3%)
≥1	138 (76.7%)
<5	125 (69.4%)
≥5	55 (30.6%)
Mean, 95% CI	4.0 (3.3–4.8)
CTCs after surgery (n=93)	
<1	41 (44.1%)
≥1	52 (55.9%)
<5	78 (83.9%)
≥5	15 (16.1%)
Mean, 95% CI	2.7 (1.7–3.2)
CTCs after surgery + chemotherapy (n=56)	
<1	19 (33.9%)
≥1	37 (66.1%)
<5	43 (76.8%)
≥5	13 (23.2%)
Mean, 95% CI	7.4 (2.9–11.8)

CTCs, circulating tumor cells.

between the two groups related to age (P=0.16), sex (P=0.72), SUVmax (P=0.81), histology (P=0.23), pathological nodal involvement (P=0.15), pathological stage (P=0.66), or pattern of relapse (P=0.58). There were no differences between the two study groups in terms of OS, with an HR of 1.05 (0.94–1.17, P=0.45), or DFS, which had an HR of 1.04 (0.94–1.15, P=0.44).

For stage IIIA, there were no significant correlations between the two groups with respect to age (P=0.456), sex (P=0.82), SUVmax (P=0.114), pathological nodal involvement (P=0.764), histology (P=0.54), or pattern of relapse (P=0.27). We found no differences between the two study groups in OS, with an HR of 0.98 (0.75–1.30, P=0.91), or DFS, which had an HR of 0.92 (0.70–1.21, P=0.56).

Relation between postchemotherapy CTCs and clinical parameters

Samples were collected during the prespecified period after

Table 3 Clinical characteristics with respect to whether patients had detectable CTCs before surgery (cut-off =1)

Clinical characteristics	CTC <1 (n=42)	CTC ≥1 (n=138)	P value
Sex, n (%)			0.243
Male	30 (71.4)	93 (67.4)	
Female	12 (28.6)	45 (32.6)	
Age, years, mean (95% CI)	65.6 (62.9–68.3)	66.4 (64.9–67.9)	0.966
SUVmax, mean (95% CI)	10.1 (7.5–12.7)	8.8 (7.8–9.9)	
Stage, n (%)			0.800
IA	14 (33.3)	38 (27.5)	
IB	9 (21.4)	35 (25.4)	
IIA	6 (14.3)	15 (10.9)	
IIB	5 (11.9)	14 (10.1)	
IIIA	8 (19.0)	36 (26.1)	
Histology, n (%)			0.742
Adenocarcinoma	21 (50.0)	78 (56.5)	
Squamous cell	16 (38.1)	47 (34.1)	
Other histologies	5 (11.9)	13 (9.4)	
Relapse, n (%)	10 (23.8)	44 (31.9)	0.487
Pattern of relapse, n (%)			0.780
Multiple	3 (42.9)	11 (45.9)	
Oligometastatic	4 (57.1)	13 (54.1)	

CTCs, circulating tumor cells; SUVmax, maximum standardized uptake value.

surgery in 56 patients.

There were no significant correlations between the two groups with respect to age (P=0.65), sex (P=0.94), SUVmax (P=0.94), histology (P=0.60), pathological nodal involvement (P=0.70), pathological stage (P=0.20), or pattern of relapse (P=0.11).

There were no significant differences in the proportions of relapsing patients between those who had one or more postchemotherapy CTCs and those with no presurgical CTCs (32.4% *vs.* 42.1%, P=0.47).

We found no differences between patients with at least five postchemotherapy CTCs and those who had no presurgical CTCs in terms of relapse (37.2% *vs.* 30.8%, P=0.67).

Change between preoperative and postoperative CTCs

The mean change in the number of CTCs over time between preoperative and postoperative samples was 2.13, with a standard deviation of 6.78.

Difference between preoperative, postoperative and postchemotherapy CTCs

The mean change in the number of CTCs between preoperative and postoperative-postchemotherapy samples was –2.74, with a standard deviation of 13.37.

Patients with significant mean change in the number of CTCs between preoperative and postoperative samples

Four patients exhibited a significant increase in CTC levels after surgery (more than 10 CTCs). Only one of them experienced a relapse and died of lung cancer. Eight patients had a significant decrease in CTC levels after surgery (more than 10 CTCs), 4 of whom (50.0%) experienced a relapse of the disease, and 3 (37.5%) died.

Discussion

In the present prospective, single-center study, with a

Table 4 Clinical characteristics according if patients had five or more CTCs before surgery

Clinical characteristics	CTC <5 (n=125)	CTC ≥5 (n=55)	P value
Sex, n (%)			0.622
Male	84 (67.2)	39 (70.9)	
Female	41 (32.8)	16 (29.1)	
Age, years, mean (95% CI)	65.5 (64.8–68.1)	65.7 (63.5–67.8)	0.585
SUVmax, mean (95% CI)	9.2 (8.0–10.4)	8.9 (7.3–10.5)	0.758
Stage, n (%)			0.144
IA	39 (31.2)	13 (23.6)	
IB	24 (19.2)	20 (36.4)	
IIA	17 (13.6)	4 (7.3)	
IIB	14 (11.2)	5 (9.1)	
IIIA	31 (24.8)	13 (23.6)	
Histology, n (%)			0.193
Adenocarcinoma	65 (52.0)	34 (61.8)	
Squamous cell	49 (39.2)	14 (25.5)	
Other histologies	11 (8.8)	7 (12.7)	
Relapse, n (%)	36 (28.8)	18 (32.7)	0.596
Pattern of relapse, n (%)			0.994
Multiple	9 (42.9)	4 (44.4)	
Oligometastatic	12 (57.1)	5 (55.6)	

CTCs, circulating tumor cells; SUVmax, maximum standardized uptake value.

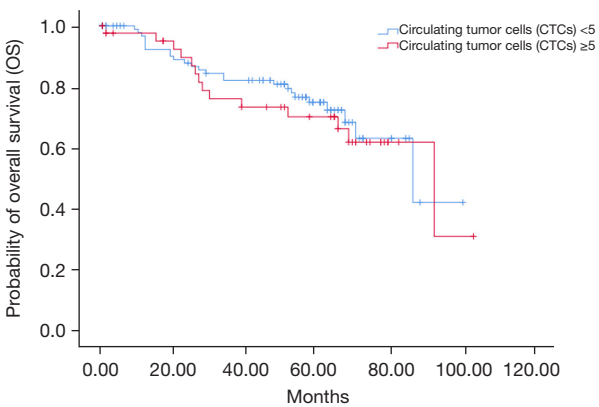

Figure 3 Overall survival for a cut-off value of 5 CTCs in presurgical samples. CTC, circulating tumor cell.

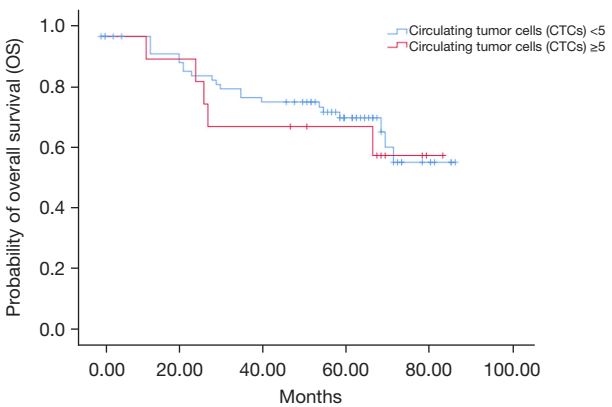

Figure 4 Overall survival for a cut-off of 5 CTCs in postsurgical samples. CTC, circulating tumor cell.

cohort of 180 patients analyzed, we did not find any significant associations between levels of CTCs before or after surgery with respect to OS or DFS.

Despite stages I and II having substantial representation, we found that 76.7% of patients had at least one CTC in peripheral blood before surgery, but only 30.6% had more

than five peripheral CTCs. This is consistent with previous studies in the perioperative setting of NSCLC, which have reported rates of 22.2–91.3%, and indicates that most patients had a measurable blood tumor burden, even in limited stages (I to III) of the disease (23-26).

When we compared the CTCs isolated before and after surgery, we found that the patients with high levels of CTCs presurgery were those with high levels of postsurgical CTCs. Neither the presurgical nor the postsurgical CTC values in our study were associated with DFS or OS. The same was found for CTC determination after completing chemotherapy, whereby patients with higher presurgery CTC levels were those with higher levels once surgery and chemotherapy had been completed.

Previous studies have addressed whether CTC isolation and its changes during the following-up period can be prognostic in terms of relapse, DFS and OS, but their results are unclear and somewhat inconsistent, at least in the localized setting of NSCLC (23-27).

CTCs have been widely studied in the locally advanced/metastatic setting, and pre-treatment levels and the variation after chemotherapy have both been linked to worse OS and PFS in several studies (28-31). Although this correlation has not been reported in all of the studies in this setting (29-31), the majority of them suggest that CTC monitoring could be a useful biomarker under such circumstances.

Although in the locally advanced/metastatic setting it is accepted that CTC monitoring during disease correlates with OS and DFS, there are fewer reports from studies in the perioperative setting and those that have been undertaken have involved relatively small numbers of patients. Bayarri-Lara *et al.* (24) prospectively assessed the prognostic significance of CTCs in 56 patients with resectable NSCLC and, similar to our study, collected samples before and after surgery. They found a significant correlation between the presence of CTCs, using a cut-off of 1 CTC after surgery, and DFS, but no correlation between presurgical CTCs and OS or DFS.

In contrast to these results, Crosbie *et al.* (25) reported a significant correlation between the presence of CTCs, using a cut-off point of 1 CTC before surgery, and 3-year DFS and OS in a small cohort of 33 patients. However, their CTC detection rate in peripheral blood was 22.2%, considerably lower than our rate, and 10% of the patients had not undergone complete resection, which makes meaningful comparisons difficult.

Using a higher cut-off value of 5 CTCs, Li *et al.* (23) demonstrated significant correlations between CTC detection before surgical resection and DFS and OS in a cohort of 23 patients.

de Miguel-Pérez *et al.* (27) also explored their prognostic value in a cohort of 97 patients, finding a significant association between DFS and detection of CTC (using a cut-off of 1 CTC) 1 month after surgery, and with detection of CTCs 6 months after surgery only for adenocarcinoma.

The largest cohort published so far in this setting to our knowledge was reported by Hofman *et al.* (26). They explored CTC isolation in 208 patients, and reported a significant correlation with OS and DFS using a cut point of 50 CTCs in peripheral venous blood, a value very different from that used in the previous report, preventing a meaningful comparison of the results.

An important finding from our study was that, despite having no detectable CTCs before surgery, up to 23.8% of patients relapsed or died from the disease, and 50.0% of the patients in our cohort who experienced a reduction of more than 10 CTCs after surgery relapsed. This suggests that CTCs may not be a reliable indicator of minimal residual disease (MRD).

There are also a few reports suggesting that CTCs have a prognostic significance in the localized setting when radiotherapy is the chosen therapeutic approach, but it is probably unwise to compare these data with results from a perioperative setting in which the entire macroscopic tumor has been removed (32,33).

In summary, some results from the various studies are contradictory in the perioperative context, suggesting that CTCs may be associated with DFS and OS. However, the studies used different cut-off points and it is not clear whether the CTCs before surgery, after surgery, or both, are of prognostic significance. Our study, which encompassed a bigger sample when using cut-off points of 1 and 5, did not find this correlation, perhaps reflecting the inconsistent prognostic significance of CTCs.

CTC detection rates differ between peripheral venous blood and pulmonary venous blood, which may imply that there is a clearance of CTCs in the microcirculation (25).

Furthermore, it is also unclear whether the isolated CTCs in the localized setting have metastatic potential, or if they are merely transient or residual tumor cells with no metastatic potential that may be cleared from blood circulation. In our study, presurgical CTC levels differed between histologies. This is consistent with previous reports, and implies that a different pattern of CTC expression may exist (34). In this line, AXL overexpression and epithelial-mesenchymal transition (EMT) activation in

CTCs of patients with localized lung adenocarcinoma have been linked to DFS and OS (27). Wan *et al.* attempted to characterize the gene expression of CTCs in the localized setting, and found that the *NOTCH1*, *IGF2*, *EGFR*, and *PTCH1* genes are very frequently mutated in more than half of these cells (35). The presence of CTCs in the pulmonary vein has also been associated with the presence of spread through air spaces (STAS), a prognostic pathological finding (36). In our study, when we analyzed the patients who experienced a sharp increase in CTCs after surgery (more than 10 CTCs), 25% died from disease relapse, but 75% of them were disease-free with a mean follow-up of 45.8 months. This led us to hypothesize that the immune system plays a critical role in the clearance of these CTCs and in the control of MRD.

A deeper understanding of the biology of these cells, which may be heterogenous, and their relationship with the immune microenvironment in the localized setting is needed to derive a more solid prognostic model.

In this context, ctDNA may be a more reliable marker for MDR, as has been suggested by prospective chemoimmunotherapeutic clinical trials such as NADIM and CheckMate816 (3,4).

The main strengths of our study are its large sample, especially in the presurgical setting, the prospective character of its design, and our decisions to include only patients with resectable NSCLC, and to perform a separate analysis of stages I to II and of stage IIIA. In addition, a CTC determination after completing surgery and chemotherapy was available in 31.1% of patients.

However, our study also has some limitations. In particular, 48.3% of patients did not have a CTC determination after surgery and the range of the collecting period (7 days to 6 months) may have been the source of some of the heterogeneity in our results.

Conclusions

CTC monitoring in the perioperative setting was not correlated with relapse, DFS or OS in our study, and therefore cannot be recommended as a reliable biomarker for MRD after surgery. A deeper understanding of the biology of CTCs and their interaction with immune system is needed to better characterize their potential prognostic value.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-827/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-827/coif). Dr. Mariano Provencio reports that has received consultant fees and support for attending meetings and/or travel from Bristol Myers Squibb, Roche, AstraZeneca, and Takeda; consultant fees from MSD; and support for attending meetings and/or travel from Boehringer Ingelheim and Pierre Fabre (Inst). VC reports that has received Payment or honoraria for lectures, presentations or speakers bureaus from Roche, BMS, MSD, Astrazeneca, Takeda, Pfizer and Lilly; support for attending meeting and/or travel: Takeda and Roche AstraZeneca; and served in Advisory board of Roche, AstraZeneca, AMGEN, BMS, Sanofi and Takeda. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by The Hospital Universitario Puerta de Hierro Ethics Committee (No. 296). Written informed consent was obtained from every patient prior to their participation.

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