STM JOURNALS

Research & Reviews: Journal of
Surgery

https://journals.stmjournals.com/rrjos

ISSN: 2319-3425 (Online)
ISSN: 2349-3712 (Print)
Volume 13, Issue 3, 2024
September—December
DOI (Journal): 10.37591/RRJoS

Research RRJoS

Utilizing Circulation-Derived Cancer Cells to Evaluate Patients with Surgically Treated Stages I–IIIA NSCLC Throughout the Preoperative Phase

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Abstract

Context: *When a tumor is considered resectable, surgery is viewed as the primary treatment approach for both early-stage and locally advanced non-small cell lung cancer. One of the most exciting areas of cancer research in the past ten years is liquid biopsy, which offers a practical non-invasive method for cancer detection and tracking. Circulating tumor cells have been linked to a worse prognosis and increased chance of relapses in various cancer types where their prognostic significance has been examined. This study aims to assess the predictive significance of circulating tumor cell identification in surgically treated patients with stage I–IIIA non-small cell lung cancer.* ***Methodology:*** *We included 180 consecutive patients with resected, pathologically confirmed stage I to IIIA non-small cell lung cancer (according to the TNM AJCC/UICC 8th edition) in our prospective, single-center study. Prior to and following surgery, the blood samples from the patients were processed, and circulating tumor cells were described. Following chemotherapy and surgery, a patient cohort had their circulating tumor cell determined. Cut-off thresholds were set for statistical analysis in circulating tumor cells 1 and 5.* ***Result:*** *Before surgery, 76.7% of the patients had at least one circulating tumor cell, and 30.6% had five or more. After surgery, 55.9% still had at least one circulating tumor cell, while 8.3% had five or more. Preoperative circulating tumor cell detection with a cut-off of 5 did not correlate with any of the following outcomes: relapse (32.7% vs. 28.8%, P = 0.596), disease-free survival (hazard ratio: 0.95, P = 0.39), or overall survival [hazard ratio: 0.99, P = 0.887]. Additionally, at a cut-off of 5, we did not observe any link between the detection of postoperative circulating tumor cells and overall survival (hazard ratio: 1.01, P=0.808), disease-free survival (hazard ratio: 0.95, P = 0.952), or relapses (26.7% vs. 29.5%, P = 0.83). The average change in the number of circulating tumor cells from before to after surgery was 2.13, with a standard deviation of 6.78.* ***Conclusion:*** *CTC monitoring in the perioperative period was not connected with recurrence, DFS, or OS in our investigation, despite the sizeable patient cohort included. As a result, it cannot be suggested as a trustworthy biomarker for minimal residual disease (MRD) following surgery.*

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Received Date: August 21, 2024
Accepted Date: October 19, 2024
Published Date: November 18, 2024

Citation: Tshetiz Dahal, Ankit Singh. Utilizing Circulation-Derived Cancer Cells to Evaluate Patients with Surgically Treated Stages I–IIIA NSCLC Throughout the Preoperative Phase. Research & Reviews: Journal of Surgery. 2024; 13(3): 17–28p.

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

INTRODUCTION

With 2.2 million new cases and 1.8 million deaths globally, lung cancer is the leading cause of cancer-related deaths and ranks as the second most common cancer for both men and women. Although the primary factor influencing prognosis is the stage of the tumor at diagnosis, less than 26% of patients with non-small cell lung cancer (NSCLC) are expected to survive five years

following diagnosis [1]. When the tumor is thought to be resectable, surgery is viewed as the cornerstone of treatment for early and locally progressed NSCLC. European guidelines recommend that patients with resected stage II or III NSCLC, as well as those with resected stage IB tumors larger than 4 cm, should receive neoadjuvant systemic therapy [2]. Neoadjuvant chemo-immunotherapy has been shown to be effective in these situations by recent studies, which also show encouraging survival rates [3, 4]. Even with the best care, 5-year survival rates for early NSCLC are still lower than those for other cancer types. This is probably because there is a post-operative risk of recurrence, with about a 25% chance of local progression and an extra 13% risk of distant relapse for stages I and II [5]. One of the most exciting areas of cancer research in the past ten years has been liquid biopsy, which offers a practical non-invasive method for cancer detection and tracking [6]. Liquid biopsy primarily examines circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), but extracellular vesicles, RNA, and tumor-educated platelets are also important biomarkers. CTCs, which are intact and often viable cells, can be identified in blood using various methods, such as antibodies targeting epithelial or mesenchymal proteins (like EpCAM, cytokeratins, vimentin, or N-cadherin), negative selection through leukocyte depletion with anti-CD45 antibodies, or based on physical properties, such as size, deformability, density, and electrical charge [7]. ctDNA is made up of tiny free nucleic acid fragments that carry tumor mutations and can be identified using novel molecular models like next-generation sequence (NGS) or traditional techniques like polymerase chain reaction (PCR) [8]. Research has examined the prognostic significance of CTCs in other cancer types, including colorectal cancer, where it has been demonstrated that these cells are linked to a worse prognosis and an increased chance of relapse [9]. To ascertain if CTC counts are associated with prognosis in lung cancer, especially in its early stages, there is insufficient prospective data, despite certain retrospective studies suggesting such a relationship [10–13]. The study's objective is to assess the predictive significance of CTC discovery in patients with stage I–IIIA NSCLC treated with surgery.

MATERIALS AND METHODS

Study Structure

Our prospective, single-center study included 180 consecutive patients with resected, pathologically confirmed stage I to IIIA NSCLC, according to the TNM AJCC/UICC 8th edition. Between 2021 and 2023, a baseline sample was drawn from radial venous blood between the time of the tumor's diagnosis and surgery. After surgery, a second sample was taken anywhere from seven days to six months later. After adjuvant chemotherapy was finished, we took a third blood sample from 56 patients (Figure 1). Every patient was tracked in order to assess their level of survival.

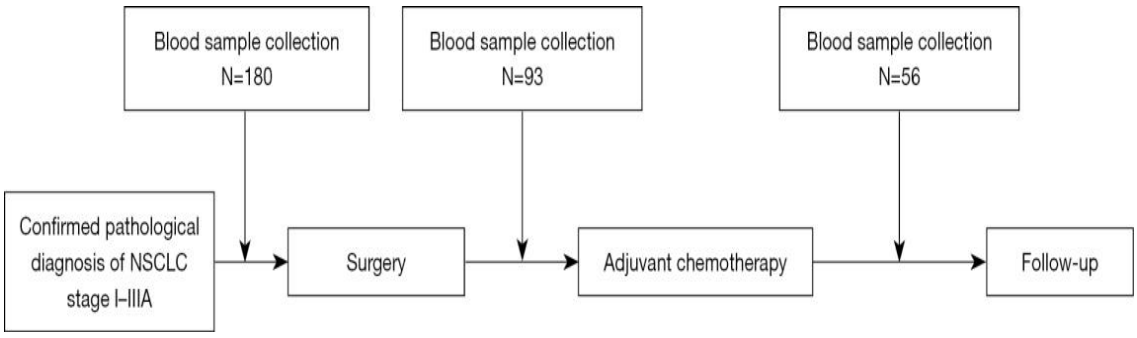

Figure 1. Timetable for collecting blood samples over the course of the investigation. NSCLC.

This research followed the 2013 revision of the Declaration of Helsinki and was approved by Lugansk State Medical University in Ukraine. Prior to their involvement, each patient provided written informed consent. The Biomedical Sciences Research Institute Ukraine's Confocal Microscopy Core Facility and Flow Cytometry Core Facility handled and examined the samples. The study's objective is to assess the predictive significance of CTC discovery in patients receiving surgical treatment for stage I–IIIA NSCLC.

Screening and Identification of Tumor Cells

Blood samples from patients were processed using the CTC isolation and characterization protocol that our group developed and that has been documented in other publications [14, 15]. In short, blood was drawn into CellSave Preservative Tubes (Veridex) for CTC analysis. The double-density gradient approach (HISTOPAQUE-1077/HISTOPAQUE-1119) was used to pre-enrich CTCs. Using EpCAM micro-beads and selective positive immuno-magnetic cell separation, CTCs were enriched [16]. An AutoMACS (Miltenyi Biotec) magnetic separator was used to purify and enrich the magnetically tagged cell suspension in a magnetic field. Reagents were added after capture such that flow cytometry and confocal imaging could identify the intracellular and extracellular phenotypic characteristics of CTCs [17]. To fluorescently label the enriched fraction, we used anti-human CD45-APC, anti-human CD326-EpCAM PE, a nuclear dye to identify live cells, and anti-cytokeratin-FITC [18]. The process of fixing methanol and washing in PBS was used to achieve intracellular staining [19]. Following an hour of room temperature incubation, samples were mounted in PBS/glycerol and quantified using confocal microscopy (Figure 2).

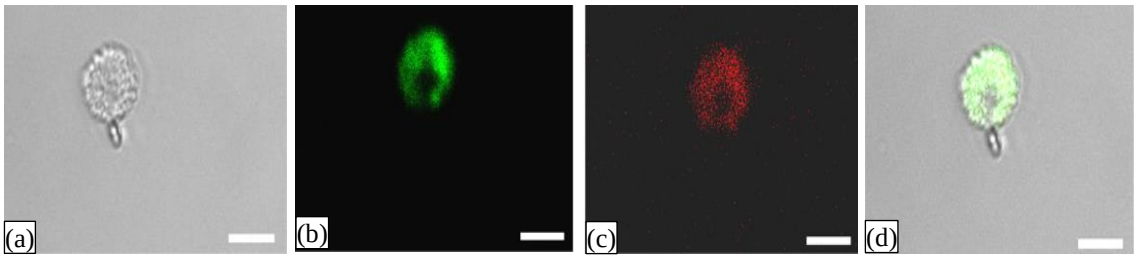

Figure 2. CTC image. (a) Phase contrast; (b) labeling with cytokeratin (green); (c) labeling with EpCAM (red); (d) merge. Bars correspond to 20 μm. CTC.

We analyzed the samples using a MACSQuant flow cytometer (Miltenyi-Biotec) equipped with three solid-state lasers, which enabled us to simultaneously assess up to ten parameters. A TCS SP5 confocal microscope (Leica Micro-systems, Wetzlar, Germany) with a 20× 0.4 lens and a 3× optical zoom was used to capture the microscopy pictures. Leica LASFlite, we used Leica Microsystems (Wetzlar, Germany) and the MACS Quantify-TM Software v2.5 (Miltenyi Biotec) to analyze the data. Based on established cut-offs from previous studies, we set thresholds for CTC detection at 1 and 5. Patients with fewer than 5 CTCs were classified as having low levels, while those with 5 or more CTCs were classified as having high levels. We then compared these two patient groups [20].

STATISTICAL ANALYSIS

IBM SPSS Statistics v. 26 was used to conduct statistical analysis. Overall survival (OS) was defined as the time from the date of surgery to death from any cause. Disease-free survival (DFS) was the time from surgery to either a confirmed relapse or death from any cause, whichever occurred first. We used ANOVA, Student's t-tests, Chi-square tests, Pearson's product correlation for group comparisons, and Kaplan–Meier survival analysis with the log-rank test for survival analysis. A p-value of less than 0.05 was considered significant. To assess changes in CTCs, we calculated the difference between preoperative and postoperative values and considered variations of ±10 as significant. Relapse was classified as either multiple or oligometastatic; oligometastatic relapse was defined as having no more than five metastases in up to three organs, including the lungs. Results were categorized into stages IA through IIB and IIIA [21, 22].

RESULTS

Patient Characteristics

With a median age at diagnosis of 66 years, 57 (31.7%) women and 123 (68.3%) males made up the 180 patients. About the histology of tumors, 35% had squamous cells, 10% had various diseases, and 55% had adenocarcinomas. 28.9% of the stages were IA, 24.4% were IB, 11.7% were IIA, 10.6% were IIB, and 24.4% were IIIA. Table 1 displays the complete findings with reference to the distribution of CTCs and clinical features.

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)

Note: CTCs; SUVmax, maximum standardized uptake value.

CTC Allotment

Table 2 displays all available data. 76.7% of patients had detectable CTCs prior to surgery, 55.9% after surgery, and 66.1% after chemotherapy and surgery were finished, assuming 1 CTC as the cut-off.

When five CTCs were considered as the cut-off, 30.6% of patients had detectable CTCs prior to surgery, 8.3% after surgery, and 23.2% after chemotherapy and surgery. The distribution of clinical features for patients with detectable CTCs before surgery, using a cut-off value of 5, is shown in Tables 3 and 4.

RELATIONSHIP BETWEEN PREOPERATIVE CTCs AND CLINICAL PARAMETERS

Regarding age (P = 0.97), sex (P=0.32), SUVmax (P = 0.73), pathological nodal involvement (P = 0.74), pathological stage (P = 0.85), and pattern of relapse (P = 0.11), we could not find any significant link between the two groups. Patients with squamous cell carcinoma were more likely than those with adenocarcinoma to have high levels of CTC (P = 0.02). P = 0.487 showed no significant

difference in the relapse rates of patients with at least one pre-surgical CTC compared to those without any pre-surgical CTCs (31.9% vs. 23.8%). When patients with five or more pre-surgical CTCs were compared to those with fewer, no significant differences were found in the rates of relapse (32.7% vs. 28.8%, P=0.596), OS (0.90–1.01, P = 0.887), or DFS (0.95–1.06, P = 0.39). OS is displayed in Figure 3.

Table 2. CTC allotment.

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)

Note: CTCs.

Table 3. Clinical characteristics with respect to whether patients had detectable CTCs before surgery.

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)	
Relapses, 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)	

Note: CTCs; SUVmax, maximum standardized uptake value; cut-off = 1.

Table 4. Clinical characteristics according to 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)	
Relapses, 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)	

Note: CTCs; SUVmax, maximum standardized uptake value.

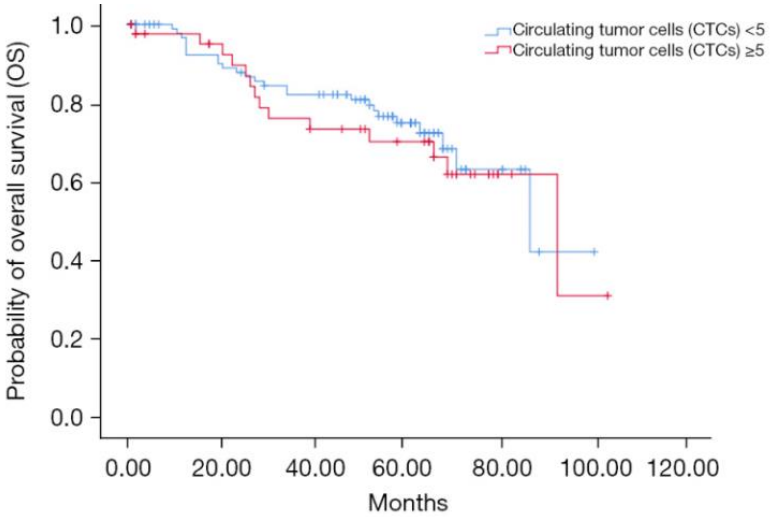

Figure 3. OS for a cut-off value of 5 CTCs in pre-surgical samples. CTC.

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), and relapse pattern (P = 0.28) did not significantly correlate between the two groups for stage I–II. When comparing the two study groups (with a cut-off of 5 CTCs), there were no differences in either OS (HR of 0.97 (0.83–1.15, P = 0.80) or DFS (HR of 0.95 (0.80–1.13, P = 0.55). In stage IIIA, we did not find any significant differences between the groups in terms of SUVmax (P = 0.74), pathological nodal involvement (P = 0.07), sex (P = 0.35), age (P = 0.47), or relapse pattern (P = 0.33).

There were different distributions of CTCs in stage IIIA adenocarcinomas compared to squamous cell carcinomas (P = 0.02). When considering a cut-off of 5 CTCs, there were no differences seen

between the two research groups for either DFS (HR of 0.94 (0.83–1.05, P = 0.29) or OS (HR of 0.99 (0.88–1.10, P = 0.84).

RELATIONSHIP BETWEEN CLINICAL FACTORS AND POSTOPERATIVE CTCs

A total of 93 individuals had samples taken during the predetermined time frame following surgery.

Regarding 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), and pattern of relapse (P = 0.33), we could not find any significant connections between the two groups. The percentages of relapsing cases did not differ between patients with no postsurgical CTCs and those with at least one (30.8% vs. 26.8%, P = 0.678).

Patients with five or more postsurgical CTCs did not differ from those with fewer than five postsurgical CTCs in terms of relapse (26.7% vs. 29.5%, P = 0.83), OS (HR of 1.01 (0.91–1.12, P = 0.808), or DFS (HR of 0.95) (0.91–1.10, P = 0.952). OS is shown in Figure 4.

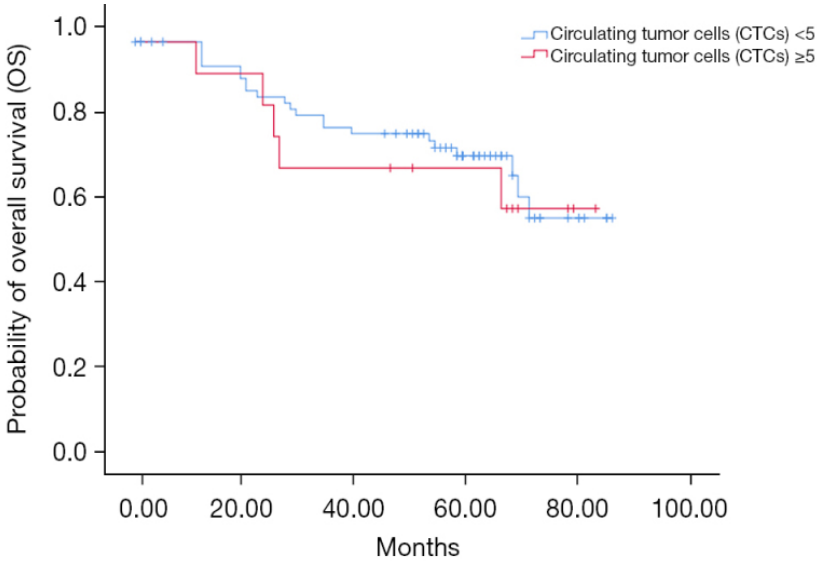

Figure 4. OS for a cut-off of 5 CTCs in postsurgical samples. CTC.

For stage I–II, there were no significant correlations between the two groups with respect 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). Similarly, there were no differences between the two research groups in OS with a hazard ratio (HR) of 1.05 (0.94–1.17, P = 0.45) or DFS with an HR of 1.04 (0.94–1.15, P = 0.44).

For stage IIIA, there were no significant correlations between the two groups regarding age (P = 0.456), sex (P = 0.82), SUVmax (P = 0.114), pathological nodal involvement (P = 0.764), histology (P = 0.54), or relapse pattern (P = 0.27). Additionally, OS had a HR of 0.98 (0.75–1.30, P = 0.91) and DFS had an HR of 0.92 (0.70–1.21, P = 0.56), showing no differences between the two research groups.

RELATIONSHIP BETWEEN CLINICAL INDICATORS AND CTCs FOLLOWING CHEMOTHERAPY

In 56 patients, samples were taken within the predetermined time frame following surgery. Regarding 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), and pattern of relapse (P = 0.11), there were no significant relationships found between the two groups. The percentages of patients who

relapsed and had one or more post-chemotherapy CTCs compared to those who had no pre-surgical CTCs (32.4% vs. 42.1%, P = 0.47) did not show a significant difference. Regarding relapses, there were no differences observed between patients with at least five post-chemotherapy CTCs and those without any pre-surgical CTCs (37.2% vs. 30.8%, P = 0.67).

SWITCHING BETWEEN CTCs BEFORE AND AFTER SURGERY

The average change in the number of CTCs from preoperative to postoperative samples was 2.13, with a standard deviation of 6.78. When comparing CTCs before surgery, after surgery, and after chemotherapy, the mean difference between preoperative and postoperative-post-chemotherapy samples was –2.74, with a standard deviation of 13.37.

DISCUSSION

We investigated a cohort of 180 patients in this prospective, single-center investigation, and we were unable to identify any significant correlations between the levels of CTCs prior to or during surgery with OS or DFS. We found that 76.7% of patients had at least one CTC in their peripheral blood before surgery, but only 30.6% had more than five CTCs, even though stages I and II were well represented. This indicates that a significant number of patients had detectable tumor cells in their blood, even at earlier stages (I to III) of the disease. These findings are consistent with previous studies in the perioperative setting of NSCLC, which reported detection rates ranging from 22.2% to 29.3%. Patients with high presurgery CTC levels were also found to have high post-surgical CTC levels when we compared the CTCs isolated before and after surgery. In our investigation, there was no correlation seen between pre-surgical and postsurgical CTC values and either DFS or OS. Similar results were observed for CTC determination following chemotherapy completion: patients with greater presurgery CTC levels also had higher levels following surgery and chemotherapy. Whether CTC isolation and its alterations over the course of the follow-up period can be predictive of relapse, DFS, and OS has been the subject of prior research; however, the findings, at least when pertaining to NSCLC in the localized setting, are ambiguous and inconsistent [23–27]. Numerous studies have examined CTCs in the context of locally progressed or metastatic disease, and pre-treatment levels, as well as the variation following chemotherapy, have been connected to poorer OS and progression-free survival [28–31]. While not all studies in this context have reported this link [29–31], most of them imply that CTC monitoring might be a helpful biomarker in such situations.

While it is widely acknowledged that CTC surveillance during the disease is correlated with OS and DFS in the locally advanced/metastatic condition, there are few data from studies conducted in the perioperative setting, and those that have been conducted have involved comparatively small numbers of patients. While it is widely acknowledged that CTC surveillance during the disease is correlated with OS and DFS in the locally advanced/metastatic condition, there are few data from studies conducted in the perioperative setting, and those that have been conducted have involved comparatively small numbers of patients. Like our study, Bayarri-Lara et al. [24] collected samples both before and after surgery while evaluating the prognostic value of CTCs in 56 patients with resectable NSCLC. They found a significant link between the presence of CTCs after surgery and DFS, but there was no significant association between pre-surgical CTCs and OS or DFS. In contrast, Crosbie et al. [25] observed a strong connection between 3-year DFS and OS and the presence of CTCs using a cut-off of 1 CTC before surgery in a smaller cohort of 33 patients. However, comparisons are difficult because 10% of their patients did not have a complete resection, and their CTC detection rate of 22.2% was notably lower than ours. Li et al. [23] found significant correlations between DFS and OS and CTC detection before surgery in a group of 23 patients, using a higher cut-off of 5 CTCs.

In a cohort of 97 patients, de Miguel-Pérez et al. [27] also investigated their prognostic significance and discovered a significant correlation between DFS and CTC detection (with a cut-off of 1 CTC) 1 month following surgery, and with CTC detection 6 months following surgery exclusively for

adenocarcinoma. To our knowledge, Hofman et al. reported the biggest cohort published thus far in this situation [26]. Using a cut-off of 50 CTCs in peripheral venous blood – a threshold much higher than in previous studies – they examined CTCs in 208 patients and found a significant association with OS and DFS, which made statistical comparison difficult. A notable finding from our study was that 50.0% of patients who had a reduction of more than 10 CTCs after surgery experienced a relapse, and up to 23.8% of patients died from the disease despite having no detectable CTCs before surgery. This implies that CTCs might not be a trustworthy measure of the disease's minimal residual disease (MRD).

Certain reports also indicate that CTCs are predictive in the localized setting when radiation therapy is selected, but it is probably not a good idea to compare these results with outcomes from a perioperative setting where the entire macroscopic tumor has been removed [32, 33]. In conclusion, there are inconsistent findings from the different studies regarding CTCs, DFS, and OS in the perioperative setting. It's uncertain whether CTCs detected before surgery, after surgery, or both are predictive, as the studies employed different cut-off values. This link was not found in our study, which included a larger sample when utilizing cut-off values of 1 and 5, which may reflect the variable prognostic importance of CTCs.

The difference in CTC detection rates between pulmonary and peripheral venous blood may indicate that CTCs are being cleared from the micro-circulation [25]. Additionally, it is unknown if the isolated CTCs in the localized situation are essentially temporary tumor cells that may be removed from blood circulation or if they have the capacity to spread meta-statically. Pre-surgical CTC levels varied throughout histologists in our investigation. This is in line with earlier findings and suggests that there might be another pattern of CTC expression [34]. DFS and OS have been associated with AXL overexpression and activation of epithelial-mesenchymal transition (EMT) in CTCs from patients with localized lung adenocarcinoma [27]. To explore gene expressions in CTCs within a localized setting, Wan et al. found that more than half of these cells had mutations in genes like NOTCH1, IGF2, EGFR, and PTCH1 [35]. Additionally, spread through air spaces (STAS), a predictive pathological feature, has been linked to the presence of CTCs in the pulmonary vein [36]. In our study, after an average follow-up of 45.8 months, 25% of patients with a rapid increase in CTCs (more than 10 CTCs) died from disease relapse. However, 75% of these patients remained disease-free. This prompted us to postulate that the immune system is essential for both controlling MRD and eliminating these CTCs.

To develop a more reliable prognostic model, a deeper comprehension of the biology of these cells – which may be heterogeneous – and how they interact with the immunological milieu in the confined setting is required. According to prospective chemoimmunotherapeutic clinical trials like NADIM and CheckMate816, ctDNA might be a more accurate marker for MDR in this situation [3, 4]. Our study's primary strengths include its high sample size, particularly in the pre-surgical environment, its prospective design, the fact that we limited the trial to patients with respectable NSCLC, and the fact that we separately analyzed patients in stages I through II and in stage IIIA. Furthermore, 31.1% of patients had a CTC determination accessible following surgery and treatment. Our study does, however, have certain shortcomings. Specifically, 48.3% of patients did not receive a CTC determination following surgery, and some of the variability in our data may have come from the collection period's 7-day to 6-month duration.

CONCLUSIONS

As a result, CTC monitoring in the perioperative setting cannot be suggested as a trustworthy biomarker for MRD following surgery because it was not connected with recurrence, DFS, or OS in our study. A more comprehensive comprehension of the biology of CTCs and their relationship with the immune system is required to more accurately assess their potential prognostic significance.

Ethical Statement

The authors have full responsibility for the work, guaranteeing that any doubts about the authenticity or correctness of any portion are duly examined and settled.

The LSMU Ethics Committee (No. 324508) approved this study, which was conducted in accordance with the 2013 revision of the Declaration of Helsinki. Prior to their involvement, each patient provided written informed consent.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Funding Statement

This study did not receive any funds.

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