Predictive &Prognostic Value of Circulating Tumor Cells in Small-Cell Lung Cancer

Osama Mohamed[1], Ola Khorshid[1], Abeer Bahnasy[2], Maha Yehia[1], Ahmed Magdy Rabea[1], Eman Desouky[3]

Abstract

Background: Small cell lung cancer (SCLC) has a very aggressive course with high propensity for metastasis. The median overall survival (OS) is about 12–20 months with only 6% to 12% of patients surviving for 5 years after diagnosis even in early stages of the disease. Currently, there are no validated biomarkers to follow the disease activity During treatment of SCLC. Detection of circulating tumor cells (CTCs) is a novel laboratory technique currently in use to determine response to therapy and to predict prognosis in breast, lung, colorectal and prostate cancer. We hypothesize that CTCs may be a valuable biomarker for therapeutic response, and survival in patients with SCLC. The aim of our study is the identification of predictive & prognostic value of Circulating tumor cells in small-cell lung cancer and its effect on the clinical response, PFS and OS.**Design and Methods:** this prospective study included 51 patients with small cell lung cancer (SCLC) diagnosed at the National Cancer Institute, Cairo University in the period from 2019 till 2021 with follow up period of 18 months. Detection of Circulating Tumor Cells (CTCs) by flow cytometry: CTCs assessed in 7.5ml of fresh blood at presentation Clinical data were obtained from patients' files. Aim of work: Assess the relation between clinical response to treatment in SCLC patients and circulating tumor cells (CTCs), Assess the relation between circulating tumor cells (CTCs) and progression- free survival (PFS) and overall survival (OS) in SCLC patients.**Results**: Out of 51 patients of SCLC, all patients with Pretreatment CTCs level ≤5 developed clinical response (17 patients). Six months PFS for patients with pretreatment CTCs level ≤5 was 94.1% compared to 50 % for patients with pretreatment CTCs level >5. while One-year overall survival for patients with pretreatment CTCs level ≤5 was 94.1% compared to 40.8 %for patients with pretreatment CTCs level >5. This is shows significant Correlation between Pretreatment CTCs level with cut-off value of 5 cells with clinical response progression-free survival and overall survival.**Conclusions:** There was a significant correlation between CTCs and clinical outcome which will allow us to use CTCs as predictive and prognostic markers in the management of SCLC but more clinical trials are needed to prove our findings.

KeyWords: Circulating tumor cells, clinical response, Progression-free survival, overall survival, Small-cell lung cancer.

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Correspondingauthor: Osama Mohamed **E-mail:**osamamohimen@yahoo.com

Affiliations:

1. Medical Oncology Department, National Cancer Institute, Cairo University, Cairo, Egypt
2. Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt
3. Epidemiology and biostatistics Department, National Cancer Institute, Cairo University, Cairo, Egypt.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Introduction:

Lung cancer remains the leading cause of cancer death worldwide. Approximately 10% to 15% of lung cancers are of small cell histology. Small cell lung cancer (SCLC) has a very aggressive course with high propensity for metastatic spread. The median overall survival (OS) is about 12–20 months with only 6% to 12% of patients surviving for 5 years after diagnosis even in early stages of the disease **(Herbst et al., 2008),** Most patients with SCLC have a history of tobacco smoking. The traditional staging system developed by the Veteran's Administration Lung Cancer Study Group divided patients according to the extent of disease into two stages **(Mountain et al., 2000),** Limited disease (LD) is confined to one hemithorax with regional lymph node metastasis and can be encompassed within a single radiation port. While extensive-stage disease is disease beyond the ipsilateral hemithorax, including malignant pleural or pericardial effusion or hematogenous metastases. Approximately 60% to 70% of patients have extensive disease (ED) at initial diagnosis. **(Kalemkerian et al., 2013),** SCLC is initially very sensitive to chemotherapy, with 60% to 90% of patients with LD-SCLC responding to first-line therapy, and 40% to 70% of patients achieving a complete response (CR). Response rates are lower (40% to 70%) for patients with ED-SCLC and the median OS is below one year. Patients relapsing or progressing after first-line chemotherapy have a poor prognosis. Median survival is 2 to 3 months for patients who do not receive second-line therapy. Second-line chemotherapy produces tumor responses in the range of 15% to 28%. **(Owonikoko et al., 2012),**the presence of CTCs has been demonstrated in the blood of patients with various solid tumors. **(Allard et al., 2004),** Their presence has been associated with poor outcome in metastatic breast, colorectal, prostate, gastric, and non-SCLC. (Cohen et al., 2008), In SCLC, the presence of ≥2 CTCs/7.5 ml of peripheral venous blood was found in 75% of patients with both LD and ED. (Naito et al., 2012), the presence of CTCs may rather reflect the metastatic potential of the tumor and therefore may correlate better with survival than the bulk of disease as reflected by tumor imaged with computed tomography (CT). **(Budd et al., 2006) (De Giorgi et al., 2010)**

Aim of work

Primary objectives: Assess the relation between clinical response to treatment in SCLC patients and circulating tumor cells (CTCs).

Secondary objectives: 1) Assess the relation between circulating tumor cells (CTCs) and progression- free survival (PFS)and overall survival (OS) in SCLC patients.

Patients and Methods

This prospective study included 51 patients with small cell lung cancer (SCLC) diagnosed at the National Cancer Institute, Cairo University in the period from 2019 till 2021 with follow up period of 18 months.

Inclusion criteria: 1)Patient must have histological or cytological proof of SCLC ;2)limited and extensive stage,3) Measurable disease, 4)Patient at least 18 years old,5) Patient with a performance status of (ECOG Scale) ≤ 2,6)No other concurrent or previous malignancies, 7)Patient with adequate bone marrow function, (WBC count ≥3.0 x109 /L, ANC ≥ 1.5 x109 /L platelet count ≥100 x109 /L, hemoglobin level ≥ 9 g/,8)Patient with adequate liver function; serum bilirubin < 1.5 X ULN, ALT and AST levels < three times normal values; ALT and AST levels < five times normal limits allowed in patients with known liver metastases, ,9)Patient with adequate Kidney function; plasma creatinine level < 1.5 times normal value and Ejection fraction ≥ 50 %,10)No other malignancies.

Exclusion Criteria:1) Patient with a second malignancy,2) Patients enrolled in another running clinical trial at the time of the study,3) Age: < 18 years, 4)ECOG PS 3-4, 5) Pregnant or lactating females.

Study Assessment:

Pretreatment evaluation included: Pretreatment assessment including complete medical history and physical examination, vital signs, performance status (ECOG), and complete blood count with differential and full biochemical panel including liver and renal function tests, radiological evaluation including CT brain, chest, abdomen, and pelvis, Imaging such as bone scan… will be done if indicated.

During treatment evaluation included: Medical history and physical examination every 3 weeks, Patients will be treated according to national

cancer institute protocols, CBC and chemistry every 3 weeks, CT chest, abdomen and pelvis after 3 cycles, and Adverse events evaluation before each cycle will be performed according to NCI toxicity criteria.

Detection of circulating tumor cells (CTCs)

1) Blood collection: Peripheral blood samples (7.5 ml; each) were obtained from all patients at presentation 2) Samples were obtained at the middle of vein puncture after discarding the first 0.5 ml to avoid skin plug contamination.

3) Detection of Circulating Tumor Cells (CTCs) by flow cytometry (CTC assay). using CK (a pan epithelial marker, positive selection) and CD45 (negative selection). One tube will be directly used (without enrichment) to separate the peripheral blood mononuclear cells (PBMCs) by gradient density centrifugation using Ficoll-Hypaque 1077 (Sigma). The separated cells will be stained with CK-FITC and CD45-TRITC according to manufacturers' protocols and acquired in the FCM (1X106-1X1012 cells/sample). CTCs are defined as being CD45-/CK+. Their number will be determined and considered as the number of CTCs in this sample. Each sample will be analyzed three times and the mean will be taken and expressed as the number of CTCs in the blood sample.

4)Cells were then acquired in the FCM and counted using the Cell Quest software. The number of CD45-/CK+ was considered as the number of CTCs in the sample.CTCs were defined as cells lacking CD45 and expressing CK.

5) A cut-off of 2+1CTCs/7.5 ml will define the test as positive.

Post treatment evaluation included: Medical history and physical examination every 2-4 months for 2 years, CT chest, abdomen and pelvis every 2-4 months for first 2 years.

Response evaluation

According to RECIST as follows: A complete response (CR) is defined as complete disappearance of all known disease determined by two observations not less than 4 weeks apart. a partial response (PR) means 30% or greater reduction of the product of the perpendicular diameters of all measurable lesions. Stable disease (SD) defined as less than 30% reduction or less than 20% increase in tumor size, progressive disease (PD) is defined as an increase of more than 20% in the product of the perpendicular diameters of all measurable lesions or the appearance of new lesions, PFS will be determined from the start of chemotherapy until tumor progression or loss of follow-up. OS will be measured from the start of the first chemotherapy until death or loss of follow-up.

Ethical consideration: IRB approval, all patients signed an informed consent that approved by the Respective institutional review boards with confidentiality of patient's data.

Statistical Methods:

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) v. 21. Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between the 2 groups with respect to normally distributed numeric variables were done using the t-test. Non normally distributed numeric variables were compared by Mann-Whitney test. For categorical variables, differences were analyzed with X^2 (chi square) test and Fisher's exact test when appropriate. Stepwise logistic regression was done to give adjusted odds ratio and measure magnitude of the effect of different factors on the response status. Kaplan and Meier procedure was used to estimate the overall survival rates and progression free rates and comparisons between the different prognostic factors were done using the Log rank test, all p-values are two-sided. P-values < 0.05 were considered significant.

Results

Table (1): Clinico-pathologic characteristics

		Number (%)
Gender	Male	46(90.2)
	Female	5(9.8)
Age (yrs)	Median	61
	Range	(36-77)
Stage	ED	34(66.7)
	LD	17(33.3)
RESPONSE	NO response	16(31.3)
	response	35(68.6)
Response	CR	9(17.6)
	PR	26(51)
	SD	4(7.8)
	DP	12(23.2)
1ST LINE OF TTT	CIS/EP +RTH	16(31.4)
	CARBO/EP+RTH	1(2.0)
	CIS/EP	26(51)
	CARBO/EP	8(15.7)
2NDLINE	NO	39(76.5)
	IRINOTECAN	3(5.6)
	CIS/EP	4(7.8)
	CARBO/EP	1(2)
	PACLITAXEL	4(7.8)
Pretreatment CTCs	≤5	17(33.3)
	>5	34(66.7)

LD: Limited Disease, **ED:** Extensive Disease

	Median	Minimum	Maximum
Pretreatment CTCs	19	1	1108

Table (1) summarizes patient's characteristics with regard to: Gender, age, stage, primary treatment, RTH, 2nd line, pretreatment CTCs.

As shown in table (1):

Our study included 46 males (90.2%) and 5 females (9.8%), and the median age of patients was 61 years (range from 36-77 years).

Seventeen patients (33.3%) who presented with Limited Disease(cT1a-4N0-3M0) While 34 patients (66.7%) presented with ED: Extensive Disease (cT1a-4N0-3M1).

Twenty-six (9.8%) patients in the study were subjected to chemotherapy cisplatin, etoposide, while 16(31.4%) of patients received concurrent cisplatin, etoposide with RTH, 8 (15.7%) patients received carboplatin, etoposide. and 1(2%) patient received concurrent carboplatin, etoposide with RTH.

Seventeen-patients (33.3%) patients received concurrent chemoradiotherapy While 34 patients (66.7%) did not.

Twelve (23.5%) patients were eligible to receive 2nd line chemotherapy, irinotecan, taxol, patient showed PD after six months of first line rechallenged by 2nd line cisplatin etoposide.

Overall Response rate

Out of 51 patients of the study 9 (17.6%) patients had complete clinical response in (CR) compared to 26 (51%) of cases had partial response (PR). While 4 patients (7.8%) had stationary disease (SD), and 12 (23.5%) patients had progressive disease (PD).

Pretreatment CTCs. (Baseline CTCs level)

Out of 51 patients, 34 patients (66.7%) had >5 Circulating tumor cells, while 17 patients (33.3%) had ≤5 CTCs.

The median of baseline CTCs level was 19 (1-1108).

The mean of baseline CTCs level was 117 with SD (244).

Table (2): Correlation between Pretreatment CTCs and clinical response.

		Response		p value
		no-response **(SD, PD)**	response **(CR, PR)**	
CTCS	≤5	0	17(100%)	**<0.001**
	>5	16(47.1%)	18(52.9%)	
CTCS	Median (range)	77(7-1037)	6(1-1108)	**0.001**

As shown in table (2), 17(100%) of patients with Pretreatment CTCS level ≤5 developed clinical response (in the form of CR or PR).

Eighteen (52.9%) of patients with Pretreatment CTCs level >5 developed clinical response (in the form of CR or PR).

Sixteen (47.1%) of patients with Pretreatment CTCS level >5 had no response in the form of (SD and PD). This shows statistical significance with (P value <0.001).

The median of Pretreatment CTCS level without clinical response was 77(7-1037) while the median of Pretreatment CTCS level with clinical response was 6 (1-1108). This shows statistical significance with (P value = 0.001).

Other Factors affecting clinical response.

Age Table (3):

		response		p value
		no-response **(SD, PD)** n=16	response **(CR, PR)** n=35	
Age	**Mean ±SD**	64.9±4.9	56.1±9.2	**0.001**

SD: standard deviation

As shown in table (3), the mean age of patients showing clinical response is 56.1 (SD±9.2). while Mean age of patients without clinical response was 64.9 years (SD±4.9), This shows statistical significance with (P value =0.001).

Stage Table (4):

		Response		p value
		no-response **(SD, PD)** n=16	response **(CR, PR)** n=35	
Stage	**LD**	3(17.6%)	14(82.4%)	**0.135**
	ED	13(38.2%)	21(61.8%)	

LD: Limited Disease, **ED:** Extensive Disease

As shown in table (4), 82.4% of patients who presented with LD showed clinical response in comparison to 61.8% of patients who presented with ED: Extensive Disease. with (P value =0.135).

Table (5): First line of treatment

	response		p value
	no-response (SD, PD) n=16	response (CR, PR) n=35	
Cis/vp	13(38.2%)	21(61.7%)	0.030
Cis/vp+RTH	3(17.6%)	14(82.3%)	

As shown in table (5), 21 (61.7%) of patientswho received cisplatin, etoposide, showed clinical response, while 14 (82.3%) patients received concurrent cisplatin, etoposide with RTH developed clinical response. This shows statistical significance (P value = 0.030).

Survival analysis

Table (6): Progression free survival for the population of the study

	PFS%				
N	3months	6m	1yr	1.5yrs	Median PFS (Months)
51	92.2	64.7	45.1	43.0	9.0(3.8-14.2)

As shown in table (6), and **figure (1),** the 1.5-year progression free survival for the whole group of patients was 43%.

One-year progression free survival for the whole group of patients was 45.1%.

Median PFS of the study was 9.0 months with range of (3.8-14.2)

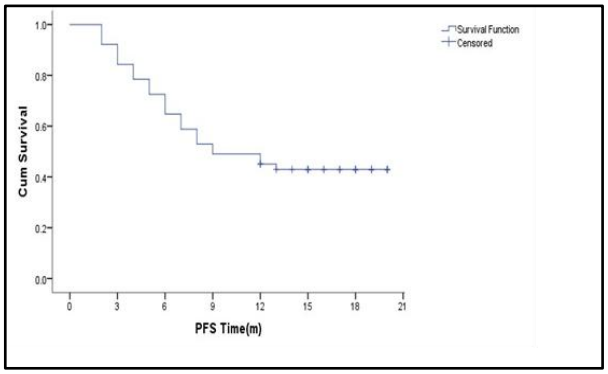

Figure (1): Progression free survival for the population of the study

Table (7): Correlation between Pretreatment CTCs and PFS.

		PFS%				Median (Months)	
Factors	n	3 months	6m	1yr	1.5yrs	(95%CI)	P value
Pretreatment CTCs							
≤5. 17 response	17	100	94.1	82.4	82.4	NR	<0.001
>5 18response- 16 no response	34	76.5	50	26.5	22.7	6(4.1-7.9)	

As shown in Table (7) and Figure (2),

Six months Progression free survival for patients with pretreatment CTCs level ≤5 was 94.1% compared to 50 %for patients with pretreatment CTCs level >5 while One year Progression free survival for patients with pretreatment CTCs level ≤5 was 82.4% compared to 26.5%for patients with pretreatment CTCs level >5 which showed statistical significance (P-value <0.001).

Eighteen months Progression free survival for patients with pretreatment CTCs level ≤5 was 82.4% compared to 22.7% for patients with pretreatment CTCs level >5. With median PFS of 6 months (range 4.1-7.9) for CTC level >5 while not reached for CTC level ≤5, This showed statistical significance (P-value <0.001).

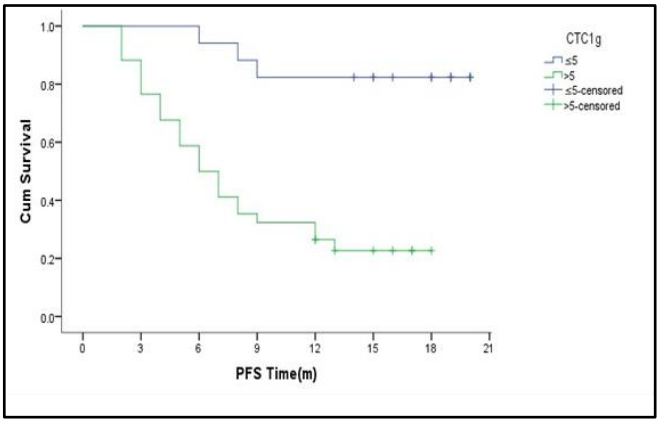

Figure (2): Correlation between pretreatment CTCs level and PFS.

Table (8): Other Factors affecting Progression free survival.

		PFS%				Median (Months)	
Factors	n	3 months	6M	1yr	1.5yrs	(95%CI)	P value
All	51	92.2	64.7	45.1	43.0	9.0 (3.8-14.2)	NA
Age (yrs.)							
≤60	25	96.0	84.0	68.0	68.0	NR	**<0.001**
>60	26	73.1	46.2	23.1	NR	6(3.5-8.5)	
Stage							
LD	17	94.1	88.2	76.5	76.5	NR	0.001
ED	34	79.4	52.9	29.4	26.1	7.0(5.1-8.9)	
RTH							
NO	34	79.4	52.9	29.4	26.1	7.0(5.1-8.9)	
YES	17	94.1	88.2	76.5	76.5	NR	0.001

Table (8) summarizes Other Factors affecting Progression free survival with regard to: age, Gender and stage.

As shown in table (8), and figure (3), one-year PFS for patients above the age of 60 was 23.1% compared to 68.0 % for patients below the age of 60 years. While 18 months PFS for patients below the age of 60 was 68.0% while not reached (NR) for patients above the age of 60 years, with median PFS of 6 months (range,3.5-8.5) for patients >60 years while not reached for patients ≤60, This showed statistical significance (P-value <0.001).

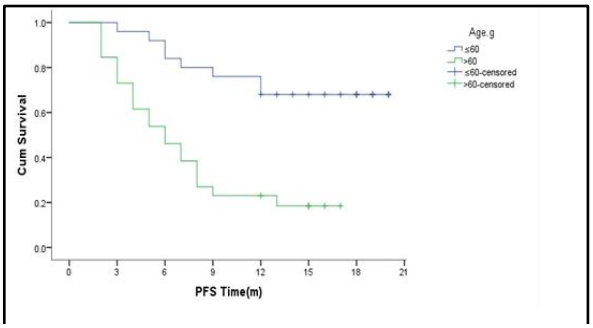

Figure (3): PFS in relation to age

As shown in table (8), and figure (4), 1year PFS for patients who presented with LD: was (76.5%) in comparison to (29.4%) of patients who presented with ED, while 18 months PFS for patients who presented with LD was (76.5%) in comparison to (26.1%) of patients who presented with ED, with median PFS of 7 months (range,5.1-8.9) for patients with ED while not reached for with LD, this showed statistical significance (P-value = 0.001).

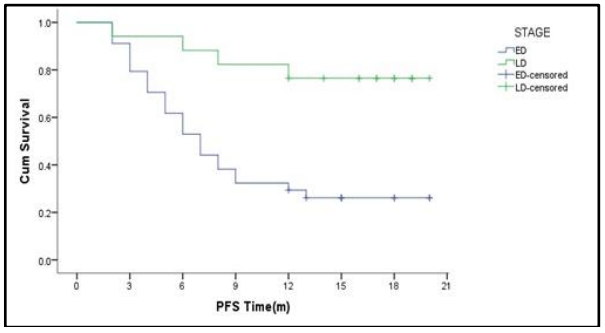

Figure (4): PFS in relation to stage.

As shown in table (8), and figure (5), one-year PFS for patients with LD who received concurrent cisplatin, etoposide with RTH was (76.5%), With median PFS of 7 months (range,5.1-8.9) for patients who received CRT while not reached for who did not, this shows statistical significance (P-value = 0.001)

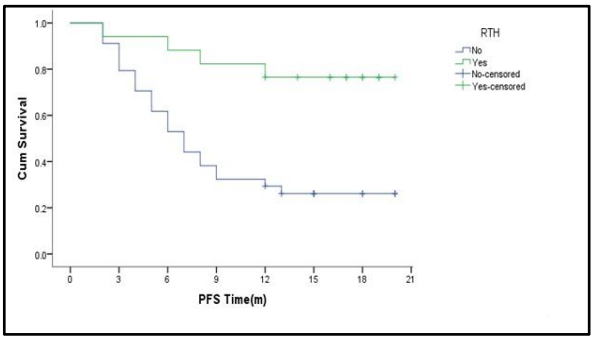

Figure (5): Correlation between CONCURRENT CRT in LD and PFS.

Table (9): Overall survival for the population of the study.

N	Overall survival %					
	3months	6m	1yr	1.5yrs	Median (Months)	P value
51	98.0	78.4	58.6	56.1	NR	NA

As shown in table (9), and figure (6), the 1.5-year overall survival for the whole group of patients was 56.1%.

One-year overall survival for the whole group of patients was 58.6%.

Three months overall survival for the whole group of patients was 98.0

The median overall survival of population of the study not reached.

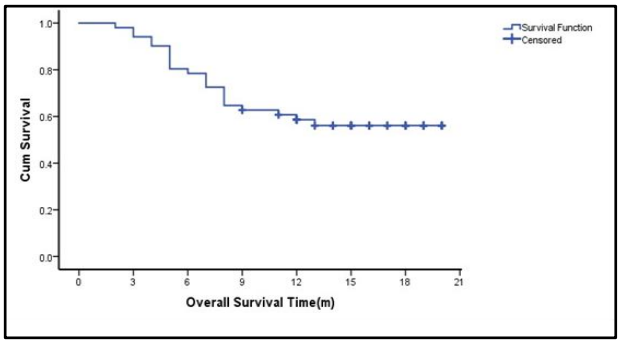

Figure (6): Overall survival for the population of the study

Table (10): Correlation between Pretreatment CTCs and overall survival.

Factors			overall survival %				Median (Months)		
			n	3 months	6m	1yr	1.5yrs	(95%CI)	P value
Pretreatment CTCs									
≤5.	17 response		17	100	100	94.1	94.1	NR	**<0.001**
>5	18response- 16 no response		34	91.2	67.6	40.8	35.7	8.0(4.2-11.8)	

As shown in Table (10) and Figure (7).

One-year overall survival for patients with pretreatment CTCs level ≤5 was 94.1% compared to 40.8%for patients with pretreatment CTCs level >5. Eighteen months' overall survival for patients with pretreatment CTCs level ≤5 was 94.1compared to 35.7 % for patients with pretreatment CTCs level >5. With median OS of 8 months (range,2.4-11.8) for CTC level >5 while not reached for CTC level ≤5, This showed statistical significance (P-value <0.001).

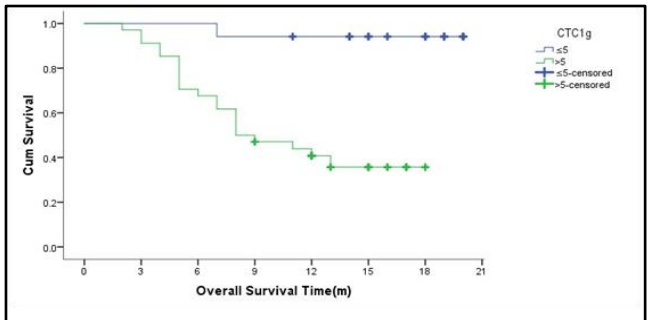

Figure (7): Correlation between pretreatment CTCs and OS.

Table (11): Factors affecting overall survival.

		Overall survival %				Median (Months)	
Factors	**n**	**3 months**	**6M**	**1yr**	**1.5yrs**	**(95%CI)**	**P value**
All	51	98.0	78.4	58.6	56.1	NR	NA
Age (yrs.)							
≤60	25	100	100	84.0	84.0	NR	<0.001
>60	26	88.5	57.7	34.6	NA	7.0(5.0-9.0)	
Stage							
LD	17	100	100	82.4	82.4	NR	0.007
ED	34	91.2	67.6	46.9	42.6	9.0(2.8-15.2)	
RTH							
NO	34	91.2	67.6	46.9	42.6	9.0(2.8-15.2)	0.007
YES	17	100	100	82.4	82.4	NR	

As shown in table (11), and figure (8), one-year OS for patients above the age of 60 was 34.6% compared to 84.0 % for patients below the age of 60 years, with median OS of 7 months (range,5-9) for patients >60 years while not reached for patients ≤60, while 18months OS for patients below the age of 60 was 84.0 % while not reached (NR) patients above the age of 60 years, with median OS of 7 months (range,5-9) for patients >60 years while not reached for patients ≤60, this showed statistical significance (P-value <0.001).

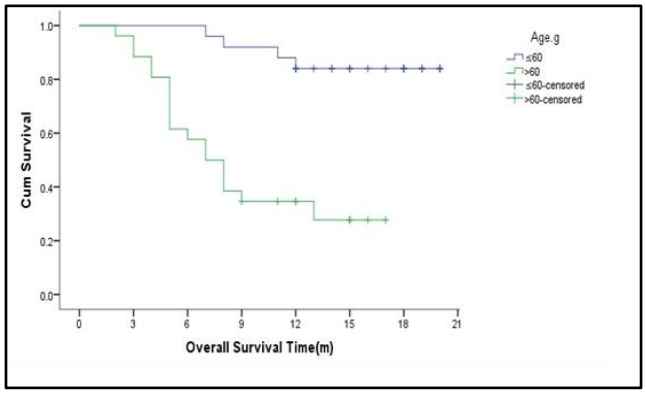

Figure (8): OS in relation to age.

As shown in table (11), and figure (9), one-year OS for patients who presented with LD was (82.4%) in comparison to (46.9%) of patients who presented with ED. This showed statistical significance, while Eighteen months OS for patients who presented with LD was (82.4%) in comparison to (42.6%) of patients who presented with ED, with median OS of 9.0 months (range,2.8-15.2) for patients with ED while not reached for patients who presented with LD. This showed statistical significance (P-value <0.001).

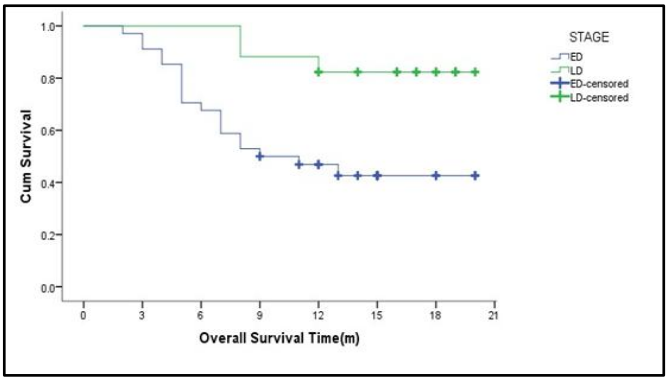

Figure (9): OS in relation to stage.

As shown in table (11), and figure (10), one-year OS for patients with LD who received CRT was (82.4%) in comparison to (46.9%) for who did not, with median OS of 9 months (range,2.8-15.2) for patients who received CRT while not reached for those who did not, this shows statistical significance with (P-value = 0.007).

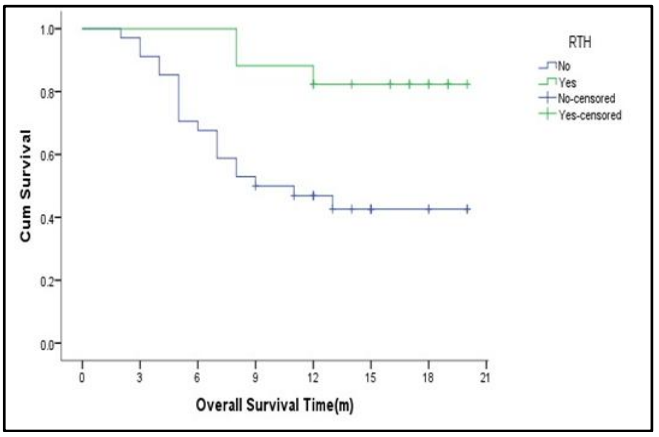

Figure (10): Correlation between Correlation between CONCURRENT CRT and OS

Table (12): Correlation between Pretreatment CTCs and Clinical response, PFS, OS.

		response (CR, PR) n=35	1yr PFS	1yr OS	P value
CTCS	≤5	17	82.4%	94.1%	<0.001
	>5	18	33.1%	44.8%	

As shown in Table (12) One-year PFS for patients showed clinical response with pretreatment CTCs level ≤5 was 82.4% compared to 33.1% for patients with pretreatment CTCs level >5 with clinical response. This showed statistical significance (P-value <0.001).

One-year overall survival for patients showed clinical response with pretreatment CTCs level ≤5 was 94.1% compared to 44.8% for patients with pretreatment CTCs level >5 with clinical response. This showed statistical significance (P-value <0.001).

Discussion

Small Cell Lung Cancer (SCLC) is an aggressive disease accounting for about 14% of all lung cancer cases **(NCCN 2022)**

In SCLC, it is important to determine whether the cancer is limited or at an extensive stage. Limited-stage cancer, which is potentially curable, is treated with chemotherapy and radiation, with surgical resection reserved for selected patients with stage I disease. Extensive-stage cancer is incurable; systemic chemotherapy is used to improve quality of life and prolong survival. **(Kalemkerian, et al., 2017)**

The high metastatic potential of the disease is due to the dissemination of tumor cells through the hematogenous and/or the lymphomatous vasculature. The presence of tumor cells in the peripheral blood (circulating tumor cells; CTCs) and bone marrow aspirates (disseminated tumor cells; DTCs) has already been described in cancer patients. **(Hartkopf et al. 2014)**

In SCLC patients, the detection of CTCs before the initiation of treatment as well as post-treatment and at the time of clinical relapse has been shown to be associated with a worse overall survival **(Naito et al. 2012)**

Mortality of SCLC remains high; even in patients with LD, 5-year survival is only ~10% (maximum 26%). This is due to metastases in many organs and perhaps to circulating tumor cells (CTCs) that originate from detachment of the primary tumor mass and migration of tumor cells to secondary sites via the lymphatic and blood system. **(Simon et al. 2007)**

In SCLC, the presence of ≥2 CTCs/7.5 ml of peripheral venous blood was found in 75% of patients with both LD and ED **(Naito et al. 2012),**The presence of CTCs may rather be a reflection of the metastatic potential of the tumor and therefore may correlate better with survival than the bulk of disease as reflected by tumor imaged with computed tomography (CT) **(Giorgi et al. 2010)**

In this study, the predictive value of CTCs for progression-free survival (PFS) and overall survival (OS) was studied. Strategies such as biomarker development to accurately monitor therapeutic responses, detect early progression, and predict clinical outcomes are powerful tools to help us further understand the complex biology of cancer and to determine clinical responses.

Currently, there are no validated biomarkers to follow the disease activity during treatment of SCLC. Detection of circulating tumor cells (CTCs) is a novel laboratory technique currently in use to determine response to therapy and to predict prognosis in breast **(Cristofanilli, et al. 2004)** colorectal **(Cohen et al. 2008)** and prostate cancer **(de Bono et al. 2008)** It is also present in lung cancer patients.

We hypothesize that CTCs will be a valuable biomarker for therapeutic response, and survival in patients with SCLC.

For this purpose and for the identification of Predictive & Prognostic value of Circulating tumor cells in small-cell lung cancer and its effect on the clinical response, PFS and OS. This prospective study included 51 patients with small cell lung cancer (SCLC) diagnosed at the National Cancer Institute, Cairo University in the period from 2019 till 2021 with follow up period of 18 months.

In our study, the threshold for prognostic significance was 5 CTCs/7.5 mL of blood, but this threshold is different among studies, as **Hiltermann et al. (2012)** tried using 2 and 5 CTCs/7.5 mL and showed that a threshold of ≥2 CTCs/7.5 mL of blood at baseline and after 1 cycle of chemotherapy were predictive of survival in patients with SCLC.

Hou et al.(2012) showed that >50 CTCs/7.5 mL of blood at baseline and changes in CTCs numbers after chemotherapy were associated with SCLC prognosis.

Naito et al. (2012) using the Cell Search system, showed that a threshold of ≥8 CTCs/7.5 mL of blood was of prognostic significance in SCLC.

Several factors could explain these discrepancies among studies, including the study population, the sample size, and the methods for measuring CTCs. This discrepancy was noted among studies about other types of solid tumors Additional studies,

preferentially multicenter and with a large sample size, will be necessary to determine the best CTCs cutoff point for prognosis of SCLC.

In our study, mean age of patients showed clinical response is 56.1years (SD±9.2), While Mean age of patients without clinical response is 64.9 years (SD±4.9) which showed statistical significance (P value =0.001). While in the study of **(Hiltermann et al., 2012)** there was no significant associations between response and age.

In this study, (82.4%) of patients who presented with LD showed clinical response in comparison to (61.8%) of patients who presented with ED (P value =0.135). While in the studies of **(Hiltermann et al., 2012)** and **(Igawa et al. 2014)** had showed statistical significance with (P value =0.009), and (P value =0.023) respectively.

Our study revealed statistically significant correlation between patients with pretreatment CTCs level ≤5and clinical response as 17 (100%) patients with Pretreatment CTCS level ≤5 developed clinical response (in the form of CR or PR),While18 (52.9%) patients with pretreatment CTCs level >5 developed clinical response (in the form of CR or PR),sixteen (47.1%) patients with pretreatment CTCs level >5 had no response in the form of (SD and PD). This showed statistical significance (P value <0.001).

Median pretreatment CTCs level without clinical response was 77(7-1037) while median of Pretreatment CTCs level with clinical response was 6(1-1108). This shows statistical significance with (P value = 0.001)

Our study revealed statistically significant correlation between Pretreatment CTCs and Progression free survival as One-year progression free survival for patients with pretreatment CTCs level ≤5 was 82.4 % compared to 26.5%for patients with pretreatment CTCs level >5. This showed statistical significance (P-value <0.001).

The current study revealed statistically significant correlation between patients showed clinical response with pretreatment CTCs level ≤5 and Progression free survival as One-year PFS was 82.4% compared to 33.1% for patients with pretreatment CTCs level >5 with clinical response, this showed statistical significance (P-value <0.001).

While 18 months Progression free survival for patients with pretreatment CTCs level ≤5 was 82.4% compared to 22.7% for patients with pretreatment CTCs level >5. With median PFS of 6 months (range 4.1-7.9) for CTC level >5 while not reached for CTC level ≤5. This showed statistical significance (P-value <0.001). Which is consistent with the results in the studies of **(Messaritakis et al., 2017), (Wang et al., 2017), (Igawa et al. 2014), (Zhang et al. 2014).**

In the study of **(Messaritakis et al., 2017)** Patients with a high number of CTCs had a significantly shorter median PFS compared to patients with a low number of CTCs irrespectively of the time of CTCs enumeration (high versus low CTCs number at baseline 6.0 versus 7.9 months respectively. (p=0.001).

While in the study of **(Wang et al., 2017)** baseline CTCs patients were categorized into a favorable and unfavorable group (<2 CTCs vs. ≥2 CTCs). Patients with unfavorable CTCs numbers had a significantly shorter median PFS (6.055 months) than patients with <2 CTCs/7.5 mL of blood (median PFS, 10.670 months) (p = 0.008).

In the study of **(Igawa et al. 2014)** 21 patients with CTCs counts of <2 cells/7.5 ml at the baseline exhibited a significantly longer median survival time (14.8 months; than the group of nine patients with a CTCs count of ≥2 cells/7.5 ml (3.9 months (P=0.007).

Zhang et al. (2014)presented a meta-analysis of seven studies conducted in 440 patients diagnosed with SCLC which supported the prognostic significance of CTCs. They concluded that the presence of CTCs (≥2) was significantly associated with reduced PFS (P<0.0001).

Our study revealed statistically significant correlation between Pretreatment CTCs and overall survival as one-year overall survival for patients with pretreatment CTCs level ≤5 was 94.1% compared to 40.8%for patients with pretreatment CTCs level >5. This showed statistical significance (P-value <0.001).

Our study revealed statistically significant correlation between patients showed clinical response with pretreatment CTCs level ≤5 and overall survival as One-year OS was 94.1% compared to 44.8% for patients with pretreatment CTCs level >5 with clinical response, this showed statistical significance (P-value <0.001).

While 18 months overall survival for patients with pretreatment CTCs level ≤5 was 94.1compared to 35.7 % for patients with pretreatment CTCs level >5. With median OS of 8 months (range,2.4-11.8)

for CTC level >5 while not reached for CTC level ≤5. This showed statistical significance (P-value <0.001). Which is consistent with the results in the studies of **(Messaritakis et al., 2017), (Igawa et al. 2014). (Naito et al.2012), (Zhang et al. 2014), (Cheng et al 2016).**

In the study by **Messaritakis et al., (2017)**, patients with a high CTCs number had a significantly decreased median OS compared to patients with a low CTCs number (high versus low CTCs number at baseline: 8.4 (95% CI: 3.2±7.5) versus 21.7 (95% CI: 15.6±27.7) months (p<0.001).

While in the study by **Igawa et al. 2014**, 21 patients with CTCs counts of <2 cells/7.5 ml at the baseline exhibited a significantly longer median survival time (14.8 months; than the group of nine patients with a CTCs count of ≥2 cells/7.5 ml (3.9 months (P=0.007).

Naito et al. (2012), using the maximal hazards ratio, identified a threshold of ≥8 CTCs, detected by Cell Search, as significantly predictive of OS (HR =3.5; 95% CI, 1.45–8.6; P=0.0014), with 78% of patients that had <8 CTCs at baseline surviving one year compared with only 31.6% of those with ≥8 CTCs detected.

Zhang et al. (2014) presented a meta-analysis of seven studies conducted in 440 patients diagnosed with SCLC which supported the prognostic significance of CTCs. They concluded that the presence of CTCs (≥2) was significantly associated with reduced OS (HR =1.9; 95% CI, 1.19–3.04; Z=2.67; P<0.0001)

Cheng et al.(2016) identify significant CTCs thresholds in their study of 91 treatment naive SCLC patients' randomized patients to two different chemotherapy regimens. They concluded that 10 CTCs detected by Cell Search appeared to be the optimal cut off for predicting PFS and OS. Multivariate analysis demonstrated that baseline CTCs count was prognostic for OS (P<0.0001) and that <10 CTCs at baseline and disease progression predicted a significantly improved median OS.

Conclusion

A Significant correlation was found between pretreatment CTCs level ≤5 in SCLC and its effect on the clinical response, PFS and OS, Potential clinical value of circulating tumor cells as a biomarker for following disease activity, and as an analytic method that can be easily incorporated into a routine diagnostic approach.

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