

Prognostic Value of CYFRA 21-1 and Carcinoembryonic Antigen in Non-Small Cell Lung Cancer

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Abstract

Background: The diagnostic value of serum tumor markers, such as cytokeratin 19 fragment (CYFRA 21-1) and carcino embryonic antigen (CEA) in non-small cell lung cancer (NSCLC) has been established. Only few studies have focused on the prognostic values of these two markers.

Objective: This study was designed to verify the prognostic significance of serum CYFRA 21-1 and CEA assay in patients with NSCLC.

Methods: The study population comprised of 40 patients of NSCLC (30 males and 10 females) with a mean age of 62.3yrs. Out of forty, twenty two had an adenocarcinoma and 18 had squamous cell carcinoma. Seven patients were at stage II, 24 were at stage III and 9 were at stage IV. None of the patients received any previous treatment. Chest computed tomography (CT) scan was done on baseline and every 2 months frequency to assess the objective radiological response. Twice serum samples were collected, initial collection was done before the beginning of treatment and the other collection was done after the second cycle of first line chemotherapy. Analysis was performed for CYFRA 21-1 and CEA using an enzyme immunoassay (EIA). Fifteen healthy volunteers with similar age and sex as the study population were selected and were used as a control group.

Results: The study revealed that 80.8% sensitivity was observed both for CYFRA 21-1 and CEA as a predictor of favourable radiological response. The cut-off values used were 10.40 ng/ml for CYFRA 21-1 and 9.30 ng/ml for CEA respectively. Univariate regression analysis identified 3 fold improved survival for the patients with post treatment CYFRA 21-1 < 10.4 ng/ml (P=0.001) and CEA < 9.3 (P=0.001). Performance status < 2 (P=0.01) and an early stage of NSCLC (P=0.03) were also found as significant independent factors associated with improved survival.

Conclusion: Comparable satisfactory results were found for both CYFRA 21-1 and CEA after 2 cycles of chemotherapy as prognostic markers for radiological response and survival outcomes in NSCLC.

Keywords: Non-small cell lung cancer; CYFRA 21-1; CEA; Radiological response; Prognostic value; Survival

Introduction

Lung cancer is among the most prevalent and lethal cancers worldwide where non-small cell lung cancer comprises approximately 85% of lung cancer cases [1].

Long-term survival with this clinical condition is poor and 5-year survival rates ranges between 7% and 15%. This is due to the fact that most patients are diagnosed in early stages where the option of surgical treatment (which is to date the most effective therapeutic strategy) no longer exists [2]. However, the majority of patients are diagnosed at more advanced stages of the disease when surgery is no longer possible. Such patients are candidates for chemotherapy which is associated with high toxicity and high cost but with limited efficacy. An objective response to chemotherapy is a surrogate marker of clinical benefit, because it is associated with a better survival outcome [3].

The best response to chemotherapy is usually achieved within 3 to 4 courses and continuation of treatment beyond the fourth cycle is not justified in the absence of a response [3,4]. Therefore, monitoring objective response after chemotherapy is essential for assessing prognosis and planning further treatment.

However, objective tumor response assessment in lung cancer is often difficult and requires repeated computed tomography (CT) scans which are costly and time consuming. Sophisticated strategies to assess the effect of chemotherapy have been explored in the recent times.

In particular, it has been reported that a decrease in the standardized uptake value of [¹⁸F] fluorodeoxyglucose by positron emission tomography (PET) may be used as an early predictor of chemotherapy outcome. However, this method appears to be costly and restrictedly applicable [5].

The development of easier and less expensive tools to monitor the effects of chemotherapy in patients with advanced NSCLC would be extremely valuable. Serum tumor markers may be used for this

purpose. In advanced prostate and ovarian cancer, the roles of prostate specific antigen (PSA) and CA125 in predicting response to the treatment and survival outcome have been clearly established [6,7].

Among the available serum markers, carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA 21-1) have been tested in NSCLC, particularly for prognosis and follow-up [8,9].

Cytokeratins are polygenic polypeptide family that constitutes the main component of keratin filaments which are crucial part of the cell cytoskeleton. There are 20 different cytokeratins with molecular weights ranging from 40 to 70 kilo daltons (kDa). These different cytokeararins are classified according to their isoelectric point into acid (type I) and basic (type II) type. Low molecular weight cytokeratins are found in simple epithelium whereas heavy molecular weights are found in epidermis [10].

Cytokeratin (CK) 19 belongs to the type I cytokeratin and has the lowest molecular mass (40 kDa) among the CKs. It is expressed in the unstratified or pseudostratified epithelium lining of the bronchial tree and has been reported as over expressed in many lung cancer tissue specimens [11]. CYFRA 21-1 (cytokeratin fragment 21-1) can be assayed to detect the soluble fragment of cytokeratin 19 in serum.

Other than lung cancer, CYFRA 21-1 was also found elevated in urological, gastrointestinal and gynaecological cancers and in lower amounts than malignances in various benign diseases, such as, pulmonary fibrosis and acute interstitial pneumonia. Therefore, the elevated level of CYFRA 21-1 precludes its use in screening of NSCLC. However, its measurement may be helpful in the differential diagnosis of suspicious lung masses, particularly when biopsy is not possible [12,13].

Carcinoembryonic antigen (CEA) is a monomeric, 200 kDa, oncofetal glycoprotein with a variable carbohydrate component of approximately 45-60%. Cancer tissues of various cell types may secrete large amounts of CEA into the circulation. However, in certain normal tissues as well as in benign diseases and in heavy smokers, CEA may

still be secreted in small amounts during adult life. Several studies documented the utility of this antigen in the early diagnosis of tumor recurrence in patients with colorectal and lung carcinoma [14,15].

Aim of the Work

This study was conducted to assess the value of serum CYFRA 21-1 and CEA after two cycles of chemotherapy as predictors for early response of patients with NSCLC.

Methods

This prospective study enrolled 40 patients with NSCLC who were selected from Minia Oncology Institute and Minia University Hospital (inpatient oncology department and outpatient oncology clinic) during the period of February, 2013 to March, 2014. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Minia University.

Fifteen apparently healthy individuals matching age and sex with patients were studied as a control group. Informed consents were obtained from both patients and controls.

All patients who participated in the study had a recent diagnosis of NSCLC that was confirmed based on pathological tests. The confirmation was done for 17 patients by trans bronchial biopsy. Another 17 patients were confirmed through CT guided biopsy. Four patients were confirmed by pleural fluid cytology and 2 patients through pleural biopsy. None of the patients had received previous treatment for NSCLC and none had a history of other forms of malignancies.

Clinical data was recorded including age, sex, smoking and pack-year information for all the participants. Stages of cancer according to TNM 2009 [16], histopathological type and Eastern Cooperative Oncologic Group (ECOG) performance status [17] were also studied (Table 1).

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e. g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled . Cannot carries on any self-care. Totally confined to bed or chair
5	Dead

Table 1: ECOG performance status.

All patients received 6 cycles of chemotherapy regimen. Out of all, twenty eight patients received first line chemotherapy (cisplatin plus gemcitabin). Ten patients received second line chemotherapy (navelbine plus carboplatin) treatment due to failure of first line therapy. Two patients underwent lung surgery and continued on the first line chemotherapy.

Blood samples were taken from all patients for complete blood count, renal and liver function tests. Collected serum from patients and controls were kept frozen at -70°C till the time of CYFRA (21-1)

and CEA assay. Another assay of serum CYFRA 21-1 and CEA levels was performed after 2 cycles of chemotherapy only for the patients.

Based on CT scans, objective responses were evaluated every 2 months after treatment following Response Evaluation Criteria in Solid Tumors (RECIST) protocol [18]. Outcomes were classified as complete response (CR), partial response (PR), progressive disease (PD) and no change (NC).

Complete response was defined by disappearance of all target lesions while partial response was those where at least 30% reduction

was observed in the sum of the longest diameter (LD) of target lesions without any new lesion formation. Progressive disease referred at least 20% increase in the sum of the longest diameter (LD) of target lesions or appearance of new lesions. No change condition referred to neither sufficient shrinkage nor increase to qualify for PR or PD respectively.

Survival was calculated from the date of objective radiological response after the first 2 cycles of chemotherapy to the date of last follow up (6 months) or death.

Assessments of serum CYFRA 21-1 and CEA

Serum CYFRA 21-1 and CEA levels were measured using an enzyme immunoassay kits supplied by Fujirebio Diagnostics, Sweden. The CYFRA 21-1 enzyme immunoassay is a solid phase immunoassay which is non-competitive in nature and dependent on two monoclonal antibodies (BM 19.21 and KS 19.1) that are directed against two separate antigenic determinants of soluble fragments of cytokeratin 19. In continuation, CEA EIA is also a non-competitive immunoassay based on the direct sandwich technique.

Calibrators, controls and patient samples were incubated together with biotinylated anti-CYFRA 21-1 MAb and horseradish peroxidase (HRP) labelled anti-CYFRA 21-1 MAb in streptavidin coated microstrips for CYFRA 21-1 assay. Biotinylated anti-CEA monoclonal antibody and horseradish peroxidase (HRP) labelled anti-CEA monoclonal antibody in streptavidin coated microstrips was used for CEA assay.

After washing, buffered substrate chromogenic reagent was added to each well and the enzyme reaction was allowed to proceed. During the enzyme reaction blue colour was developed in the presence of the antigen. The intensity of the colour development was proportional to the amount of CYFRA 21-1 and CEA present in the samples.

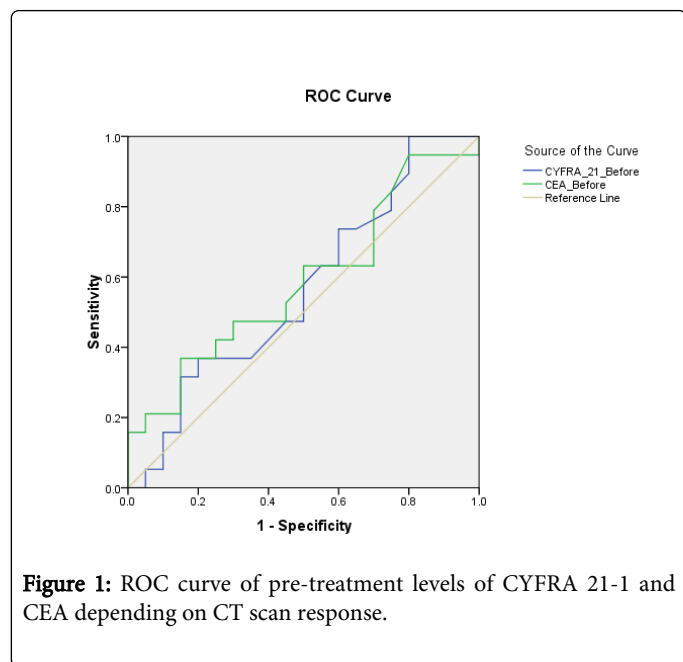


Figure 1: ROC curve of pre-treatment levels of CYFRA 21-1 and CEA depending on CT scan response.

Statistical analysis

Data was collected, coded and analysed using Statistical Package for Social Science (SPSS version 19) software. Qualitative data was presented as frequency distribution with its percentage, while

quantitative data was represented as means and standard deviation. Comparisons of qualitative data were performed using chi-squared test and student's t-test was used to compare quantitative data. ANOVA was performed depending on the need of the analysis. P-values of <0.05 was considered as the cut-off point to determine the level of significance. For calculating sensitivity and specificity and finding out the cut off points for different tumor markers, receiver operating characteristic (ROC curve) was applied.

The ROC curve analysis reveals the performance of baseline CYFRA 21-1 and CEA in predicting objective response to chemotherapy. The areas under the curve (AUC) were 0.56 (95% CI: 0.38-0.74, P=0.48) for pre-treatment CYFRA 21-1 and 0.58 (95% CI: 0.40-0.76, P=0.35) for pre-treatment CEA. CYFRA 21-1 cut-off value of 5.3 ng/ml to predict sensitivity and specificity for favorable radiological response was found as 52.6% and 50% respectively. CEA sensitivity and specificity was observed as 57.1% and 50% respectively considering a threshold value of 5.5 ng/ml (Figure 1).

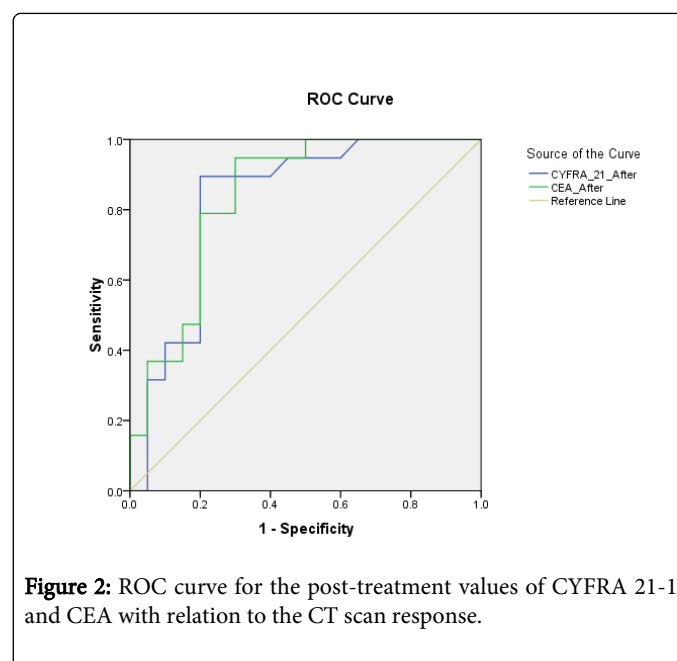


Figure 2: ROC curve for the post-treatment values of CYFRA 21-1 and CEA with relation to the CT scan response.

The ROC curve analysis for the post-treatment levels of CYFRA21-1 and CEA for responsive patients for the chest CT scan had areas under the curve (AUC) of 0.82 (95% CI: 0.68-0.96, P=0.001) and 0.83 (95% CI: 0.70-0.96, P=0.0001) respectively at cut-off values of 10.40 for CYFRA 21-1 and 9.30 for CEA. Sensitivity, specificity for CYFRA 21-1 was 80.9% and 89.5% respectively, while that of CEA was 80.8% and 78.9% respectively (Figure 2).

Results

Table 2 shows that 17.5% of NSCLC patients were at stage II (12.5% IIa and 5% IIb), 60% (42.5% IIIa and 17.5% IIIb) at stage III and 22.5% were at stage IV. It shows that both baseline CYFRA 21-1 and CEA levels are significantly higher in NSCLC patients compare to the healthy controls (P=0.001). On the other hand, CYFRA 21-1 and CEA levels of the patients after 2 cycles of chemotherapy are not statistically significant than their pre-treatment levels (P=0.37 for CYFRA 21-1 and P=0.44 for CEA).

	NSCLC Patients (N=40)	Controls (N=15)	P-value
Age (years)			
Mean ± SD	62.33 ± 8.08	58.4 ± 8.26	0.178
Gender			
Males	30 (75%)	9 (60%)	0.345
Females	10 (25%)	6 (40%)	
Smoking			
Yes	30 (75%)	8 (53.4%)	0.345
No	10 (25%)	7 (46.6%)	
Mean Pack-yr			
<10	19 (47.5%)	7 (46.6%)	0.355
≥ 10	21 (52.5%)	8 (53.4%)	
Histopathology of NSCLC			
Squamous cell carcinoma	18 (45%)	-	-
Adenocarcinoma	22 (55%)	-	-
Stages of NSCLC			
II	7 (17.5%)	-	-
III	24 (60%)	-	
IV	9 (22.5%)	-	
Objective Radiological Response			
Complete response	0	-	-
Partial response	20 (50%)	-	
No change	4 (10%)	-	
Progressive disease	16 (40%)	-	
CYFRA 21-1 level (ng/ml)			
Pre-treatment level	8.13 ± 11.92	0.56 ± 0.2	0.001
After 2 cycles of chemotherapy	7.54 ± 11.6	-	
CEA level (ng/ml)			
Pre-treatment level	28.26 ± 56.72	0.2 ± 0.13	0.001
After 2 cycles of chemotherapy	21.68 ± 41.77	-	
Survival			
Alive	26 (65%)	-	-
Dead	14 (35%)	-	

Table 2: Descriptive data recorded for the patients and the controls Data representation is in number and percentage.

Table 3 presents that level of both CYFRA 21-1 and CEA increased significantly with advanced stage of cancer and performance status. CYFRA 21-1 is significantly higher in squamous cell carcinoma while CEA is significantly higher in adenocarcinoma. On the other hand, levels of these tumor markers have no significant difference with respect to the gender of patients.

Variable	CYFRA 21-1 (ng/ml)	P-value	CEA (ng/ml)	P-value
Type of NSCLC				
Squamous cell (n=18)	16.21 ± 16.6	0.03	6.49 ± 9.3	0.01
Adenocarcinoma (n=22)	4.63 ± 3.31		60.2 ± 79.4	
Stage of NSCLC				
Stage II (n=7)	3.45 ± 4.98	0.001	3.65 ± 4.46	0.005
Stage III (n=24)	8.91 ± 9.17		12.01 ± 17.6	
Stage IV (n=9)	28.32 ± 12.58		81.4 ± 77.01	
Performance status				
<2 (n=25)	3.49 ± 2.26	0.01	3.48 ± 2.97	0.001
≥ 2 (n=15)	21.95 ± 15.02		79.89 ± 78.79	
Sex				
Males (n=30)	6.88 ± 7.49	0.44	29.01 ± 61.18	0.98
Females (n=10)	15.86 ± 19.27		25.55 ± 39.58	

Table 3: CYFRA 21-1 and CEA levels according to clinical variables.

Levels of CYFRA 21-1 and CEA in relation to objective radiological response, it was found that either pre-treatment or post-treatment CYFRA 21-1 levels had no significant change among those who partially respond or had a progressive disease detected through chest CT scan. On the other hand, post-treatment level of CEA only had a significant higher value in patients who had a progressive disease than patients who partially respond to the chest CT scan (Table 4).

	Partial radiological response (n=20)	Progressive and no change (n=20)	P-value
CYFRA 21-1 (ng/ml)			
Pre-treatment level	18.99 ± 44.87	19.97 ± 23.57	0.86
Post-treatment level	15.53 ± 49.66	26.24 ± 33.19	0.43
CEA (ng/ml)			
Pre-treatment level	32.23 ± 67.86	72.31 ± 119.74	0.20
Post-treatment level	15.00 ± 40.04	89.04 ± 145.84	0.035*

Table 4: Pre and post-chemotherapeutic CYFRA 21-1 and CEA levels with relation to the radiological response.

It was found that both pre and post-treatment values of CYFRA 21-1 and CEA were significantly higher among patients of NSCLC

who died during the follow up period than those who were alive (Table 5).

	Alive (n=26)	Dead (n=14)	P-value
CYFRA 21-1 level			
Pre-treatment	7.88 ± 10.09	36.09 ± 54.57	0.01*
Post-treatment	7.25 ± 11.79	44.84 ± 63.12	0.006*
CEA level			
Pre-treatment	17.93 ± 36.48	112.16 ± 138.56	0.003*
Post-treatment	15.26 ± 36.45	105.02 ± 136.02	0.005*

Table 5: Levels of CYFRA 21-1 and CEA with relation to survival

Discussion

Lung cancer is the major cause of cancer related death in western countries. Tumor markers are of equally significance for both researchers and clinicians to understand tumor biology and to treat patients with cancer [19]. Research on prognostic factors in non-small cell lung cancer is of great importance as it potentially leads to a better and perhaps tailored management of patients [20].

Traditionally, tumor markers measured prior to the treatment in lung cancer patients have shown higher levels than in the control groups [21]. This study has also shown that pre-treatment serum levels of CYFRA 21-1 and CEA are significantly higher in NSCLC patients than healthy controls (8.13 ± 11.92 ng/ml and 28.26 ± 56.72 ng/ml respectively) resulting in a P value of 0.001.

The observations in this study agreed with the results obtained by Abd El-Nabi et al., (6.44 ± 6.82 ng/ml) and Okamura et al., (7.9 ± 34.6 ng/ml) who earlier demonstrated that the mean CYFRA21-1 levels in patients with NSCLC were higher compare to the control groups. Moreover, this study showed results in vicinity to these earlier reports [22,23]

Sugama et al. [24] reported earlier that increased serum levels of CYFRA 21-1 in NSCLC patients were not only due to cytokeatin release as a result of cell lysis or necrosis, but also due to the degradation of cytokeatin filaments by activated protease (caspase 3) in tumor cells.

CEA was one of the first markers measured in patients with NSCLC. Elevated levels of serum CEA have been reported in 35-60% of NSCLC patient [25]. Although, CEA is mainly associated with adenocarcinoma, increased levels of CEA have been observed in 20-30% of patients with squamous cell lung cancer [26].

This study found that pre-treatment level of CEA was 28.26 ± 56.72. Brechet et al. [27] also reported CEA level of 27.1 ± 72.7 and 109 ± 404 ng/ml for respectable and unrespectable patients of NSCLC respectively. Several other reports documented lower [28] or higher [29] values of CEA than the present study in discussion.

Study conducted by Lee et al. outlined CEA level of 6.8 ± 23.1 ng/ml [28] where the patients had a resected NSCLC only. In another study, Arrieta et al. [29] documented a mean baseline CEA of 242.8 ng/ml. The high value of CEA reported by Arrieta et al. could be attributed to the fact that the majority of the studied NSCLC patients (84.4%) were in stage IV.

Elevated serum CEA levels could be found due to the antigen production by malignant cells. Excessive level of CEA leads to aberrant cell adhesion and inhibits cell apoptosis in the case of cancer. CEA level can also reflect the tumor growth, recurrence and metastasis [30].

So far, the reported results of correlation between serum CEA levels and TNM staging in NSCLC are obscure despite of extensive studies. Salgia et al. [31] stated that serum CEA levels were significantly lower in patients with early stage disease as compared to patients with unrespectable or metastatic disease. Nonaka et al. [32] reported that serum CEA level reflected tumor size, but not tumor invasion.

We also found that there was a statistically significant difference between disease stages (II, III and IV) and biomarker (CYFRA 21-1 and CEA) levels. In accordance to our results, a clear correlation between CYFRA 21-1 levels and disease stage was observed by other authors [33], suggesting that serum CYFRA 21-1 levels may reflect the tumor mass.

The relationship between serum CEA levels and tumor histology type was also studied. Some reports have indicated that CEA levels were significantly higher in patients with adenocarcinoma compared to patients with squamous cell carcinoma [31,34].

Lee et al. [28] found that CEA was mainly elevated in adenocarcinoma whereas CYFRA 21-1 in squamous cell carcinoma. This finding corresponds with the results observed in the present study.

Another important report by Lai et al. [35] suggested that the sensitivities of CYFRA 21-1 for squamous cell carcinoma, adenocarcinoma and large cell carcinoma were 62%, 39%, and 36%, respectively. Therefore, indicating that the serum level of CYFRA 21-1 in squamous cell carcinoma is significantly higher than other cell types of cancer.

Chemotherapy is one of the main methods of treatment for NSCLC. Efficacy is routinely evaluated on the basis of radiological findings. However, this is not conducive to the early detection of recurrence and metastasis. Consequently, there is growing demand for convenient tools for estimating prognosis and for detecting responsiveness to therapy in order to optimize disease management on an individual basis. Tumor markers such as CEA and CYFRA 21-1 have been studied with the purpose of early cancer detection, prognostic stratification, and monitoring of the treatment response and cancer recurrence [8].

The present study focused on the use of the levels of tumor markers as an early indicator of response to chemotherapy and prognosis for patients with NSCLC. The prognostic values were studied using ROC curves. The prognostic analysis was based on overall survival and objective radiological response.

We found that assay of CYFRA 21-1 and CEA after 2 cycles of chemotherapy had a higher sensitivity and specificity than baseline assays on the objective radiological response.

There is no unanimous opinion with respect to the prognostic value of CEA in NSCLC patients. According to Ochnio et al. [36] and Nisman et al. [37], pre-treatment CEA concentrations have a prognostic value. Although, the observations done by Shinkai et al. [38] and Buccheri et al. [39] did not confirm these data.

Pang et al. [40] evaluated the prognostic significance of multiple serum tumor markers (CEA, CYFRA 21-1, CA 19-9, CA 125 and NSE) in predicting the response for different chemotherapy regimens in the

patients with NSCLC using objective radiological response. They found sensitivity of CYFRA 21-1 and CEA were 90.0% and 80.1% respectively while detecting response to chemotherapy.

We found that after 2 cycles of chemotherapy, patients had a partial radiological response, their serum level of CYFRA 21-1 and CEA decreased by 18% and 54% respectively compare to their pre-treatment levels. In addition, those with progressive disease detected by CT scans, had an increase of 31% of CYFRA 21-1 and 23% of CEA levels than their respective pre-treatment levels (Table 4).

Variable	Survival		Odd Ratio (95% Confidence interval)	P-value
	Alive(n= 26)	Dead (n=14)		
Age				
<60	10	4	1.13(0.70-1.81)	0.73
≥ 60	16	10		
Sex				
Female	7	3	0.92(0.52-1.62)	0.76
Male	19	11		
Pathology				
Squamous	12	6	1.08(0.69-1.72)	0.76
Adenocarcinoma	14	8		
Stage				
I+II	7	0	1.82(1.33-2.51)	0.03
III+IV	19	14		
Performance status				
<2	20	5	1.98(1.03-3.80)	0.01
≥ 2	6	9		
Baseline CYFRA-21-1				
(<5.3 vs. ≥ 5.3)	14	5	1.71(0.69-4.15)	0.32
	12	9		
CYFRA-21 after treatment				
(<10.4 vs. ≥ 10.4)	19	0	3.0(1.64-5.49)	0.001
	7	14		
Baseline CEA				
(< 5.5 vs. ≥ 5.5)	14	4	2.14(0.81-5.67)	0.18
	12	10		
CEA after treatment				
(<9.3 vs. ≥ 9.3)	20	1	3.01(1.54-5.88)	0.001
	6	13		

Table 6: Logistic univariate regression analysis of clinical characteristics and survival.

Arrieta et al. [29] found reduction (≥ 14%) of serum CEA level than the baseline level after 2 cycles of treatment in advanced NSCLC cases. This was an accurate measurement of objective radiological response and correlates especially with adenocarcinoma histology. They also observed increment in serum CEA level by ≥ 18% than the baseline which was as an accurate measurement of progressive disease.

Ardizzoni et al. [41] outlined that a reduction in CEA and CYFRA 21-1 serum levels (≥ 20%) after 2 cycles of chemotherapy could be regarded as a possible surrogate marker of chemotherapy efficacy in patients with advanced NSCLC. Moreover, Nisman et al. [42] reported that declination in CYFRA 21-1 levels (≥ 35%) after two cycles of chemotherapy could be a reliable marker for treatment efficacy and survival.

Univariate analysis suggested that the clinical variables associated with significantly better survival included performance status (<2 (P=0.01)), early stage of NSCLC (P=0.03), post-treatment CYFRA 21-1 (<10.4 ng/ml (P=0.001)) and CEA level (<9.3 ng/ml (P=0.001)) (Table 6).

Barlesi et al. [43] in univariate analysis revealed that age (<65yrs (P=0.01)), PS (P<0.0001) and TNM stage (P=0.01) was having a statistical significance on prognosis. Their results showed that serum level of CYFRA 21-1 (<3.5 ng/ml (P=0.0001)) alone or combined with CEA and neuron specific enolase (P=0.0001) also showed statistically significant influence on prognosis.

Another study [44], applying univariate analysis, demonstrated that elevated CYFRA 21-1 and CEA were both unfavorable prognostic factors. On the contrary, Blankenburg et al. [45] indicated that elevation of CYFRA 21-1 and CEA were not associated with unfavorable survival.

In the present study, we used the prognostic cutoff values that yielded the best sensitivity. There is some controversy regarding the optimal prognostic cutoff point. However, the diagnostic cut off, indicating the normal upper limit for the healthy population was used as a prognostic cut off in some studies [46,47].

In conclusion, our findings suggest that assay of serum level CYFRA 21-1 and CEA early after 2 cycles of chemotherapy are simple, easy and can serve as good prognostic factors than the baseline assay for NSCLC patients. CEA behaves similarly to CYFRA 21-1 as a prognostic tumor marker, but with the advantage of being very low cost in comparison to CYFRA 21-1.

These results can be used to make early decision about further treatment for the patients with NSCLC who did not respond to the first line chemotherapy. However, further studies with larger cohorts of patient are required to verify these results.

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