Original Article

Diagnostic and Prognostic Significance of Serum Biomarkers – Serum Amyloid A and CYFRA 21-1 in Lung Cancer

Yera Dhanurdhar, Suman Kumar Jagaty[1], Saswat Subhankar[1], Debasis Behera[1]

Department of Respiratory Medicine, Hi-Tech Medical College, [1]Department of Respiratory Medicine, KIMS, Bhubaneswar, Odisha, India

Abstract

Introduction: Bronchogenic carcinoma is a leading cause of cancer-related death in men and women. Early diagnosis and treatment in these cases are essential for a better prognosis. Serum biomarkers such as serum amyloid A (SAA) and CYFRA 21-1 have generated encouraging results regarding their use in the diagnosis of these cases but data on their role in the Indian scenario are still lacking. **Aim:** The study aims to measure the levels of SAA and CYFRA 21-1 in various types of lung cancer and compare them with patients without lung cancer. It also aims to compare the values of these biomarkers before and after chemotherapy and correlate them with response to treatment. **Materials and Methods:** It was a prospective, case–control study conducted in the Department of Pulmonary Medicine, Government Medical College, Chandigarh. All histologically and/or cytologically proven lung cancer cases were included in the study group while patients with diseases other than lung cancer formed the control group. All patients were evaluated through a complete history and thorough clinical examination. Measurement of SAA and CYFRA 21-1 in blood was done by sandwich ELISA method. The patients in the study group were followed up regularly and the biomarkers were measured again after four cycles of chemotherapy. The response of tumors to chemotherapy was evaluated as per modified Response Evaluation Criteria in Solid Tumors criteria. The statistical analysis was carried out using SPSS version 19.0. **Results:** The study group and control group included 20 patients each. Hoarseness of voice and hemoptysis were significantly associated with lung cancer patients (P = 0.001 and P = 0.025, respectively). Serum levels above 8745 ng/ml for SAA and 2.55 ng/ml for serum CYFRA 21-1 were used as diagnostic biomarker in lung cancer. The serum levels of CYFRA 21-1 were found to be significantly raised in nonsmall cell carcinoma (NSCLC) in comparison to SCLC of lung. There was a statistically significant decrease in the serum levels of CYFRA 21-1 in lung cancer patients on C4 cycle of chemotherapy in comparison to C1 cycle (P = 0.014). **Conclusion:** SAA and CYFRA 21-1 could be valuable diagnostic biomarkers in lung cancer. CYFRA 21-1, in addition, could also be used as prognostic biomarker in lung cancer patients undergoing chemotherapy as it showed significant decrease after C4-cycle of chemotherapy. It can also be a potential biomarker to differentiate small cell and NSCLC.

Keywords: *Biomarkers, bronchogenic carcinoma, Cyfra 21-1, serum amyloid A*

Introduction

Lung cancer accounts for 29% of all cancer deaths.[1] While the 5-year survival rate is 70% in those diagnosed early, it becomes progressively worse with advancing stages. Unfortunately, most patients with lung cancer have advanced disease at diagnosis.[2] Computed tomography-guided fine-needle aspiration cytology (CT-FNAC) and bronchoscopy are most used for diagnosis of lung cancer. However, these procedures are not without complications. The common complications of CT-FNAC include pneumothorax, pulmonary hemorrhage, and hemoptysis.[3] The possible complications of bronchoscopy include severe bronchospasm and pneumothorax in addition to limited use in peripheral lesions.[4] Thus, efficient and noninvasive methods for diagnosis are the need of the hour. Although annual low dose CT scan can reduce mortality among high-risk smokers, evidences are insufficient to recommend it as screening tool.[5]

Research has been conducted to identify biomarkers of lung cancer and few studies have documented the role of serum amyloid A (SAA) and CYFRA 21-1 in diagnosis of lung cancer.

SAA is an acute phase protein and a family of apolipoproteins. It is induced in liver

How to cite this article: Dhanurdhar Y, Jagaty SK, Subhankar S, Behera D. Diagnostic and prognostic significance of serum biomarkers – Serum amyloid A and CYFRA 21-1 in lung cancer. Int J App Basic Med Res 2023;13:89-94.

Submitted: 27-Dec-2022
Revised: 22-Apr-2023
Accepted: 08-Jun-2023
Published: 17-Jul-2023

Address for correspondence:
Dr. Yera Dhanurdhar, Department of Respiratory Medicine, Hi-Tech Medical College, Bhubaneswar, Odisha, India.
E-mail: yeramkcgmbbs@gmail.com

Access this article online

Website: https://journals.lww.com/IJAB

DOI: 10.4103/ijabmr.ijabmr_639_22

Quick Response Code:

during tissue injury, infection, and trauma. The level of SAA increases as cells progress to dysplasia and neoplasia.[6] Many functions of SAA in context of inflammation closely resemble tumor invasion and metastasis. CYFRA 21-1 on the other hand is a cytokeratin 19 fragment expressed in unstratified or pseudostratified epithelium lining of bronchial tree and has been reported to be overexpressed in many lung cancer tissue specimens.[7,8] Data on role of these lung cancer biomarkers in Indian scenario are, however, lacking.

Aims and objectives

The study aims to measure the levels of SAA and CYFRA 21-1 in various types of lung cancer and compare them with patients without lung cancer. It also aims to compare the values of these biomarkers before and after chemotherapy and correlate them with response to treatment.

Materials and Methods

It was a case–control study conducted in the Department of Pulmonary Medicine, Government Medical College, Chandigarh.

Inclusion criteria

All patients >18 years with of age with histologically/cytologically proven diagnosis of lung cancer visiting or admitted in the Department of Pulmonary Medicine were included as "cases." Patients >18 years (age and gender matched to study group) admitted with diagnosis other than lung cancer were considered "controls."

Exclusion criteria

Patients who completed treatment for lung cancer, patients with secondary deposits in the lung with primary tumor elsewhere in the body, and pregnant and lactating mothers were excluded from the study.

Methodology

A total of 20 patients were included in each group. All the subjects undergoing the study were given necessary information and written informed consent was obtained in a standard pro forma. Complete history, thorough clinical examination, and routine blood investigations (complete blood profile, blood sugar, viral markers, liver and renal function tests, urine routine, and chest X-ray) were performed as per structured pro forma. CT of thorax, abdomen, and brain was done whenever needed. For the determination of SAA and CYFRA 21-1, the collected blood sample was centrifuged immediately, and serum separated. The serum was stored at −70°C till the time of measurement by sandwich Enzyme-linked immunosorbent assay (ELISA) method.

The patients in the study group were followed up regularly and both the biomarkers were measured after four cycles of chemotherapy. Tumor response to chemotherapy was evaluated as per modified Response Evaluation Criteria in Solid Tumors criteria.[9]

Statistical analysis

The results were expressed as the mean ± standard deviation and percentages. The cutoff value of the serum biomarkers was calculated by receiver operating characteristic curve (ROC). The Chi-square test was used to compare categorical variables. The unpaired *t*-test was used to compare two discrete variables. The one-way analysis of variance was used to compare more than two discrete variables. All statistical analyses were performed using SPSS version 19.0 (SPSS, IBM, Armonk, New York, U.S.A). $P < 0.05$ was considered significant. The study was approved by the institutional ethics committee at GMC, Chandigarh.

Results

The mean age of patients in the study and control group was 57.05 ± 7.423 years and 59.30 ± 5.904 years, respectively ($P = 0.295$). There were 16 males and 4 females (20%) in each group [Table 1].

Cough was the most common symptom among lung cancer cases while both cough and dyspnea were most common symptoms in patients with other respiratory diseases. Dyspnea and hoarseness of voice were significantly associated with lung cancer patients ($P = 0.008$ and 0.001, respectively). Hemoptysis was seen in both the groups. Smoking was associated with 90% patients in each group [Table 1].

The mean smoking index among patients of lung cancer cases and respiratory diseases 42.94 ± 9.96 and 30.94 ± 14.94 pack years, respectively ($P = 0.0049$) [Table 1].

The most common mode of diagnosis of lung cancer was bronchoscopy. In our study group, nonsmall cell carcinoma (NSCLC) was found to be the most diagnosed lung cancer and present in 14 (70%) among which squamous cell carcinoma was present in 11 cases (58%).

SAA was significantly raised in lung cancer cases in comparison to the patients with other respiratory diseases ($P = 0.001$). The median values of SAA were 69,535.00 (Interquartile range [IQR] =118,095) and 15,952.50 (IQR = 82,607.5) ng/ml, respectively, in case and control group, respectively ($P = 0.02$) [Table 1]. A value of more than 100,000 ng/ml had a sensitivity and specificity of 35% and 100%, respectively, in differentiating the cases and controls in ROC analysis (area under curve = 0.673; $P = 0.046$) [Figure 1].

The median values of serum CYFRA 21-1 level were 23.725 (IQR = 82.925) and 0.99 (IQR = 3.97) in study and control group ($P = 0.001$) [Table 1]. A value of more than 1.32 ng/ml had a sensitivity and specificity of 95% and 70%, respectively, in differentiating the cases and controls in ROC analysis (area under curve = 0.802; $P = 0.0001$) [Figure 2].

There was no significant difference in serum levels of these biomarkers in metastatic or nonmetastatic lung cancer patients ($P = 0.92$ and $P = 0.372$, respectively) [Table 2].

Table 1: Demographic, clinical, and laboratory characteristics

Characteristic	LC, *n* (%)	RD, *n* (%)	*P*
Mean age (years)	57.05±7.423	59.3±5.904	0.295
Male:female	4:1	4:1	0.5
Clinical features			
Cough	20 (100)	18 (100)	0.073
Breathlessness	19 (95)	18 (90)	0.274
Hemoptysis	12 (60)	5 (25)	0.0125
Chest pain	7 (35)	8 (40)	0.37
Hoarseness	9 (45)	0	0.00032
Fever	7 (35)	13 (65)	0.029
Weight loss	3 (15)	0	0.036
Mean SI	42.94±9.96	30.94±14.94	0.0049
Median value of SAA (ng/mL) (IQR)	69,535.00 (118,095)	15,952.50 (82,607.50)	0.02
Median value of CYFRA 21-1 (ng/mL) (IQR)	23.725 (82.925)	0.99 (3.97)	0.001

LC: Lung cancer group; RD: Respiratory diseases group; SI: Smoking index; SAA: Serum amyloid A; IQR: Interquartile range

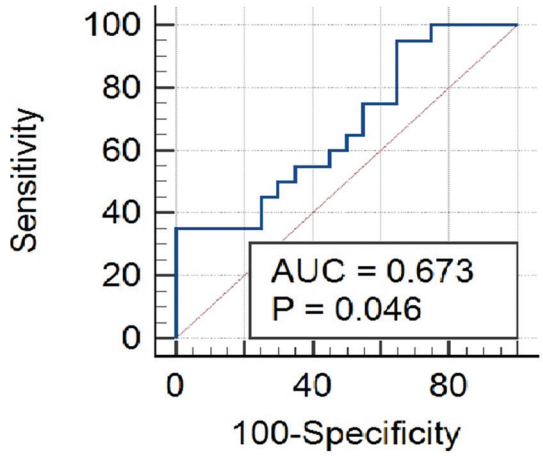

Figure 1: Receiver operating characteristic analysis of serum amyloid A levels between the lung cancer and respiratory diseases group. AUC: Area under the curve

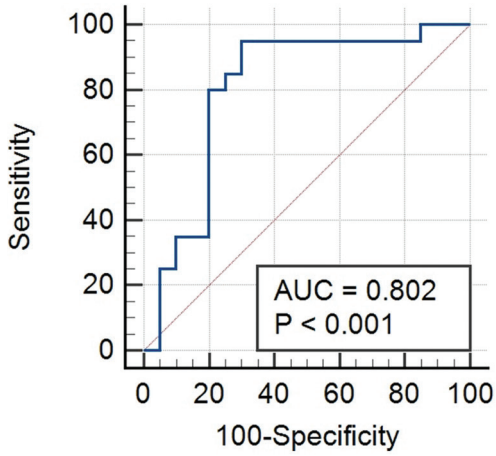

Figure 2: Receiver operating characteristic analysis of CYFRA 21-1 levels between the lung cancer and respiratory diseases group. AUC: Area under the curve

When SAA and CYFRA 21-1 were used in combination for screening of lung cancer, the sensitivity and specificity was found to be 95% and 92.5%, respectively [Table 3]. Serum CYFRA 21-1 was significantly raised in nonsmall cell lung cancer patients in comparison to small cell lung cancer patients ($P = 0.04$) [Table 4].

There was statistically significant decrease in the level of serum CYFRA 21-1 in lung cancer patients after C4-cycle of chemotherapy in comparison to the levels in patients of C1-cycle ($P = 0.014$); however, decrease in the levels of SAA values between these two groups was not found to be statistically significant [Table 5].

Discussion

The present study was conducted to evaluate the diagnostic and prognostic significance of SAA and CYFRA 21-1 as lung cancer biomarkers. In this study, 20 patients with lung cancer and 20 patients with other respiratory diseases were enrolled. The subjects enrolled were well matched for age and sex. Tumor response to chemotherapy was evaluated as per modified RECIST criteria [Table 6]. Majority of the patients in each group were male.

The most common symptoms in patients of lung cancer were cough and dyspnea. Patients with such complaints approached the health-care system often late as many patients would be treated in peripheral centers as obstructive airway disease, tuberculosis, or any other respiratory diseases, thus resulting in delay in diagnosis of lung cancer. The mean duration of development of hemoptysis and chest pain was 0.69 and 1.30 months, respectively, in patients of lung cancer. Such symptomatic patients approached for medical treatment much earlier in comparison to patients with other symptoms.

Smoking is an independent risk factor for lung cancer as well as other respiratory diseases. Higher mean smoking index was found in lung cancer patients in comparison to control group which was statistically significant.

In India, adenocarcinoma has been found to be the most common type of lung cancer.[10-12] In our study group,

Table 2: Serum biomarker levels in metastatic and nonmetastatic group

Biomarker	Group	Metastasis	Number of cases	Mean biomarker (ng/mL)	SD	SEM
SAA	LC	Yes	13	77,083.08	88,655.281	24,588.551
		No	7	124,655.71	78,746.575	29,763.408
CYFRA 21-1	LC	Yes	13	43.2	45.694	12.673
		No	7	43.21	44.559	16.842

SAA: Serum amyloid A; LC: Lung cancer group; SEM: Standard error of mean; SD: Standard deviation

Table 3: Sensitivity and specificity of serum amyloid and CYFRA 21-1 in combination

	Group		Total
	LC	RD	
Criteria (combined)			
Positive			
Count	19	3	22
Percentage within criteria (combined)	86.4	13.6	100.0
Percentage within group	95.0	7.5	36.7
Negative			
Count	1	37	38
Percentage within criteria (combined)	2.6	97.4	100.0
Total			
Percentage within group	5.0	92.5	63.3
Count	20	40	60
Percentage within criteria (combined)	33.3	66.7	100.0
Percentage within group	100.0	100.0	100.0

LC: Lung cancer group; RD: Respiratory disease group; SAA: Serum amyloid

Table 4: Mean serum level of biomarkers among small and nonsmall cell carcinoma of lung

Typing	*n*	Mean (ng/mL)	SD	SEM
C1 (CYFRA 21-1)				
SCLC	6	11.08	12.063	4.925
NSCLC	14	56.97	45.907	12.269
C1 (amyloid A)				
SCLC	6	101,308.33	87,191.505	35,595.783
NSCLC	14	90,487.14	89,148.536	23,825.948

SD: Standard deviation; SEM: Standard error mean; SCLC: Small cell carcinoma; NSCLC: Non-SCLC

NSCLC was most diagnosed (70% cases) among which, squamous cell carcinoma was present in 58% cases.

A statistically significant association was found between serum levels of SAA in patients with lung cancer and other respiratory diseases ($P < 0.001$). Cho *et al*. had reported isoform of SAA was increased by 18.22 times in lung cancer patients as compared to normal individuals. They also observed that an increase in SAA by 77% or more in lung cancer patients showed poor prognosis.[13] A similar higher level of SAA in patients with lung cancer was reported by Sung *et al*.[14]

Several studies reported a highest diagnostic sensitivity for CYFRA 21-1 in all types of lung cancer NSCLC, particularly squamous cell tumors.[15,16] In our study, serum levels of CYFRA 21-1 in lung cancer patients were found to be significantly raised ($P < 0.001$). Results of our study are in coherence with Esmat *et al*., who reported CYFRA 21-1 to be significantly elevated in all types of lung cancer with specificity of 100% and sensitivity of 65.7%. The values were significantly higher in nonsmall cell lung cancer as compared to small cell lung cancer with sensitivity of 80% and 40%, respectively.[17] Our study also found that the mean value of serum CYFRA 21-1 in NSCLC was higher as compared to SCLC ($P = 0.029$). There was no significant difference in the level of serum CYFRA 21-1 seen among metastatic and nonmetastatic lung cancer patients. However, Tan *et al*. observed that in cases of nonsmall cell lung cancer, serum CYFRA 21-1 values in NSCLC varied significantly according to Mountain's stage of disease and serum level of more than 3.6 ng/ml significantly indicated a poor survival rate.[18] The level of serum CYFRA 21-1 was significantly lowered after chemotherapy in our study. This was also observed in few other studies.[19,20] It was also observed that the serum level of CYFRA 21-1 increased with tumor–node–metastasis staging ($P = 0.0001$). On follow-up for 15–18 months, no change in CYFRA 21-1 level was observed in patients whose disease was stable, while there was significant increase in patients with progressive disease.

In our study, using the cutoff values obtained for SAA and CYFRA 21-1 in combination, their sensitivity and specificity for screening of lung cancer was found to be 95% and 92.5%, respectively. It is worth mentioning that there are no reports on use of biomarkers in combination in literature survey in lung cancer.

Limitations

The number of cases was limited because the work had to be completed in a desired frame of time. Second, the preliminary evidence showing association of various biomarkers in the screening of lung cancer patients and their prognostic significance was limited.

Conclusion

SAA and CYFRA 21-1 could be valuable diagnostic biomarkers in lung cancer. CYFRA 21-1, in addition, could also be used as a prognostic biomarker in patients of lung cancer undergoing chemotherapy. It can also be a potential biomarker to differentiate NSCLC from SCLC. However, more studies are required with larger sample size with patients of different histological types of lung cancer

Table 5: Comparison of serum biomarkers in between C1 and C4 cycle of chemotherapy in lung cancer patients

Biomarker	Paired differences					*t*	df	Significant (two tailed)
	Mean	SD	SEM	95% of the CI difference				
				Lower	Upper			
Pair 1								
C1 (CYFRA 21-1)	31.320	51.586	11.535	7.177	55.463	2.715	19	0.014
C4 (CYFRA 21-1)								
Pair 2								
C1 (SAA)	13,780.500	112,867.463	25,237.932	−39,043.099	66,604.099	0.546	19	0.591
C4 (SAA)								

C1: At cycle 1 of chemotherapy; C4: At cycle 4 of chemotherapy; SD: Standard deviation; SEM: Standard error mean; CI: Confidence Interval; SAA: Serum amyloid

Table 6: Response Evaluation Criteria in Solid Tumors criteria version 1.1

Response assessment	RECIST guideline, version 1.1
Target lesions	
CR	Disappearance of all target lesions and reduction in the short axis measurement of all pathologic lymph nodes to ≤10 mm
PR	≥30% decrease in the sum of the longest diameter of the target lesions compared with baseline
PD	≥20% increase of at least 5 mm in the sum of the longest diameter of the target lesions compared with the smallest sum of the longest diameter recorded OR The appearance of new lesions, including those detected by FDG-PET
SD	Neither PR nor PD
Nontarget lesions	
CR	Disappearance of all nontarget lesions and normalization of tumor marker levels
IR, SD	Persistence of 1 or more nontarget lesions and/or the maintenance of tumor marker levels above normal limits
PD	The appearance of 1 or more new lesions or unequivocal progression If patient has measurable disease, an increase in the overall level or substantial worsening in nontarget lesions, such that tumor burden has increased, even if there is SD or PR in target lesions If no measurable disease, an increase in the overall tumor burden comparable in magnitude with the increase that would be required to declare PD in measurable disease (e.g., an increase in pleural effusions from trace to large, or an increase in lymphangitic disease from localized to widespread)

CR: Complete response; PR: Partial response; PD: Progressive disease; FDG-PET: Fludeoxyglucose positron emission tomography; SD: Stable disease; IR: Incomplete response; RECIST: Response Evaluation Criteria in Solid Tumors

in different stages to establish the exact diagnostic and prognostic role of these biomarkers.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Minna JD, Schiller JH. Neoplasms of the Lung. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL *et al*, editors. Harrison's Principles of internal Medicine. 17th ed. United States: McGraw Hill, 2008. p. 551-62.
2. Fergusson RJ. Lung cancer. In: Seaton A, Seaton D, editors. Crofton and Douglas's Respiratory Diseases 2. 5th ed. France: Wiley India; 2011. p. 1077-80.
3. Mullan CP, Kelly BE, Ellis PK, Hughes S, Anderson N, McCluggage WG. CT-guided fine-needle aspiration of lung nodules: Effect on outcome of using coaxial technique and immediate cytological evaluation. Ulster Med J 2004;73:32-6.
4. Gupta S, Bhalotra B, Jain N. Spectrum of intrabronchial mass lesions and role of flexible bronchoscopy in their diagnosis: A series of 74 cases. Indian J Chest Dis Allied Sci 2010;52:79-82.
5. Gutfeld O, Prus D, Ackerman Z, Dishon S, Linke RP, Levin M, *et al.* Expression of serum amyloid a, in normal, dysplastic, and neoplastic human colonic mucosa: Implication for a role in colonic tumorigenesis. J Histochem Cytochem 2006;54:63-73.
6. Rastel D, Ramaioli A, Cornillie F, Thirion B. CYFRA 21-1, a sensitive and specific new tumour marker for squamous cell lung cancer. Report of the first European multicentre evaluation. CYFRA 21-1 multicentre study group. Eur J Cancer 1994;30A: 601-6.
7. Kosacka M, Jankowska R. Comparison of cytokeratin 19 expression in tumor tissue and serum CYFRA 21-1 levels in non-small cell lung cancer. Pol Arch Med Wewn 2009;119:33-7.
8. Manser R, Lethaby A, Irving LB, Stone C, Byrnes G, Abramson MJ, *et al.* Screening for lung cancer. Cochrane Database Syst Rev 2013;2013:CD001991.
9. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
10. Mohan A, Garg A, Gupta A, Sahu S, Choudhari C, Vashistha V, *et al.* Clinical profile of lung cancer in North India: A 10-year analysis of 1862 patients from a tertiary care center. Lung India 2020;37:190-7.
11. Noronha V, Pinninti R, Patil VM, Joshi A, Prabhash K. Lung cancer in the Indian subcontinent. South Asian J Cancer 2016;5:95-103.

12. Nath A, Sathishkumar K, Das P, Sudarshan KL, Mathur P. A clinicoepidemiological profile of lung cancers in India – Results from the national cancer registry programme. Indian J Med Res 2022;155:264-72.
13. Cho WC, Yip TT, Cheng WW, Au JS. Serum amyloid A is elevated in the serum of lung cancer patients with poor prognosis. Br J Cancer 2010;102:1731-5.
14. Sung HJ, Ahn JM, Yoon YH, Rhim TY, Park CS, Park JY, *et al.* Identification and validation of SAA as a potential lung cancer biomarker and its involvement in metastatic pathogenesis of lung cancer. J Proteome Res 2011;10:1383-95.
15. Kulpa J, Wójcik E, Reinfuss M, Kołodziejski L. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem 2002;48:1931-7.
16. Okamura K, Takayama K, Izumi M, Harada T, Furuyama K, Nakanishi Y. Diagnostic value of CEA and CYFRA 21-1 tumor markers in primary lung cancer. Lung Cancer 2013;80:45-9.
17. Esmat AA, Nada EG, Aael KA, Nagwa AK, Mohammed MA, Safwat SB. Evaluation of cyfra 21-1 as a diagnostic tool in lung cancer. J Appl Sci Res 2009;5:1195-201.
18. Tan Y, Zhang P, Zheng C. Usefulness of CYFRA21-1 as a tumor marker of non-small-cell lung cancer. Zhonghua Zhong Liu Za Zhi 1999;21:287-9.
19. Merle P, Janicot H, Filaire M, Roux D, Bailly C, Vincent C, *et al.* Early CYFRA 21-1 variation predicts tumor response to chemotherapy and survival in locally advanced non-small cell lung cancer patients. Int J Biol Markers 2004;19:310-5.
20. Holdenrieder S, von Pawel J, Dankelmann E, Duell T, Faderl B, Markus A, *et al.* Nucleosomes and CYFRA 21-1 indicate tumor response after one cycle of chemotherapy in recurrent non-small cell lung cancer. Lung Cancer 2009;63:128-35.