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The Role of Serum Tumor Markers in Follow-up After Surgical Treatment of Malignant Lung Tumors

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Abstract. *Aim: The aim of this study was to evaluate the utility of selected tumor markers for the detection of lung cancer recurrence during follow-up. Patients and Methods: The study group consisted of 109 patients and 109 healthy controls. The following biomarkers were selected: Carcinoembryonic antigen; cytokeratin fragment 19; neuron-specific enolase; tissue polypeptide-specific antigen; cytokeratin fragments 8, 18 and 19; insulin-like growth factor 1; pro-gastrin-releasing peptide; and 25-hydroxyvitamin D. The biomarkers were assessed individually or using a multivariate analysis. Results: Carcinoembryonic antigen [area under the receiver operating characteristics curve (AUC)=0.6857, p<0.0001] and cytokeratin fragment 19 (AUC=0.6882, p<0.0001) proved best in detecting relapse. The multivariate model indicated insulin-like growth factor 1 (p=0.0006, AUC=0.6225) as the third most useful biomarker. The multivariate model using these three markers achieved the best AUC value of 0.7730 (p=0.0050). Conclusion: We demonstrated that carcinoembryonic antigen and cytokeratin fragment 19 play a key role in the detection of lung cancer recurrence. A multivariate approach can increase the effectiveness of detection.*

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Lung cancer, one of the most common malignancies, is a disease with a poor prognosis and high mortality. Globally, it has long been one of the leading malignancies in both incidence and mortality (1, 2). The 5-year survival rate is only between 4% and 20% (3, 4), regardless of disease stage. Thus, only patients in whom the disease was diagnosed at an early stage and underwent radical surgical treatment have a real chance of longer survival.

In patients with these tumors, serum levels of certain immunochemical markers are elevated. None, however, have been evaluated as sufficiently sensitive for individual use, nor have they been identified as specific for these types of tumors (5). Our study aimed to put forward a solution to this situation by determining the sensitivity and specificity of a panel of tumor markers. The sensitivity and specificity of these markers may be useful both in making a prognosis and in detecting the recurrence of lung cancer with minimal patient burden (6).

The aim of this study was to evaluate the utility of selected tumor markers in detecting the recurrence of non-small cell lung cancer (NSCLC) in follow-up subsequent to surgery.

Patients and Methods

Groups of patients. This prospective study was conducted between April 2018 and August 2020. The study group consisted of 218

patients: 109 patients who had undergone radical surgery of NSCLC and 109 persons in the control group. The detailed characteristics of the cancer group are given in Table I. The control group consisted of patients with a non-cancer diagnosis, injury, or who had undergone surgery but did not have a positive cancer anamnesis. The patients in the control group corresponded in sex (p=0.7808) and age (p=0.9829) to the those in the cancer group.

Informed consent was obtained from each patient enrolled in the study. The study protocol was approved on 28. 6. 2018 by the Ethics Committee of the Medical Faculty and University Hospital in Pilsen (approval number 271/2018) and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws.

Diagnostic methods. Prior to the surgical treatment of lung cancer, current standard diagnostic methods were used to diagnose NSCLC: Computed tomography in combination with positron-emission tomography, computed tomography or magnetic resonance of the brain, and bronchoscopy.

Blood samples and immunochemistry methods. Peripheral venous blood (Greiner Bio-one Company, Kremsmünster, Austria) was collected using the VACUETTE system as follows: one day before surgery, 7 days after surgery, 3 months after surgery and the last sample at 1 year after surgery. Only one blood collection was performed for those of the control group.

The serum levels of the following biomarkers were determined: Carcinoembryonic antigen (CEA) using the chemiluminescence assay ACCESS CEA (Beckman Coulter, Inc., Brea, CA, USA) using a DxI 800 (Beckman Coulter, Inc.); neuron-specific enolase (NSE) using chemiluminescent assay LIAISON NSE (DiaSorin, Saluggia, Italy) in a LIAISON XL (DiaSorin); soluble cytokeratin 18 fragment (CYFRA 21-1) and pro-gastrin releasing peptide (pro-GRP) using chemiluminescent assays ARCHITECT CYFRA 21-1 and ARCHITECT pro-GRP (Abbott Laboratories, Libertyville, IL, USA) in an ARCHITECT i1000 SR (Abbott Laboratories); tissue polypeptide-specific antigen, soluble cytokeratin 18 fragment (TPS) and soluble cytokeratin fragments 8, 18 and 19 (MonoTotal) using the immunoradiometric assays TPS IRMA and MonoTotal IRMA (Immunotech, Prague, Czech Republic) in a Stratec SR 300 (Stratec SE, Birkenfeld, Germany).

Methods of surgical treatment. The surgical approach was posterolateral thoracotomy in all cases. The standard surgical procedure was anatomical lung resection in the range of at least a lobectomy and, depending on the size and location of the tumor, possibly also a bi-lobectomy or a pneumonectomy. An integral part of each operation was systematic nodal dissection, performed according to the established scheme of The International Association for the Study of Lung Cancer from 2009 (7).

All patients in the group underwent radical surgery, *i.e.,* complete resection of the tumor and descending lymph nodes (so-called R0 resection). Recurrence was defined as the recurrence of a tumor after radical surgery, and which had not been demonstrated by available diagnostic methods. We defined tumor progression as a worsening of a permanently present cancer but we did not have such patients in the group, because they were all radically operated on, so for some time they were free of cancer and new finding of tumor was therefore always a recurrence of the disease, not progression.

Table I. *Characteristics of the patient group (n=109).*

Variable		Value
Gender, n (%)	Male	66 (60.6)
	Female	43 (39.4)
Age, years	Median (IQR)	68 (40-80)
Histology of NSCLC, n (%)	Adenocarcinoma	54 (49.5)
	Squamous cell carcinoma	40 (36.7)
	Others	15 (13.8)
Type of operation, n (%)	Lobectomy	95 (87.1)
	Bi-lobectomy	9 (8.3)
	Pneumonectomy	5 (4.6)
Pathological stage, n (%)*	IA1	6 (5.5)
	IA2	29 (26.6)
	IA3	16 (14.7)
	IB	13 (11.9)
	IIA	6 (5.5)
	IIB	18 (16.5)
	IIIA	18 (16.5)
	IIIB	3 (2.8)
Involvement of intrathoracic lymph nodes, n (%)	Overall	25 (22.9)
	N1	13
	N2	4
	N1+N2	8
Adjuvant therapy, n (%)	None	71 (65.1)
	Chemotherapy	31 (28.5)
	Chemotherapy+radiotherapy	7 (6.4)

IQR: Interquartile range. *TNM classification (8).

Statistical methods. Statistical analysis was performed using S.A.S. software (Statistical Analysis Software release 9.4; SAS Institute Inc., Carry, NC, USA). Basic Descriptive statistics for numerical data (mean±standard deviation, or median with lower and upper quartile for non-normally distributed parameters) and categorical data (absolute and relative frequencies) are presented. Receiver operating characteristics (ROC) analysis, as well as univariate and multivariate logistic regression were used to compare malignant lung tumor and control groups. Cut-off values were calculated at the 95% level of specificity. The Wilcoxon two-sample test was used for comparison of individual parameters and the Spearman rank correlation coefficient was used to measure the correlation of individual parameters with stage. A p-value of less than 0.05 indicated statistical significance. The disease-free interval (DFI), calculated from the day of surgery, was analyzed using a Cox regression model with baseline tumor markers as covariates. A Cox regression model with time-dependent covariates (individual tumor markers during follow-up) was used to assess DFI.

Results

A comparison of the results for the patient and control groups is shown in Table II. The following tumor markers were significantly higher in the group of patients with NSCLC compared to the control group: CEA (p=0.0001), CYFRA 21-1 (p=0.0001), NSE

Table II. *Comparison of the results of analysis tumor markers for the patient and healthy control groups.*

Analyte	Group	Mean	Median	Lower quartile	Upper quartile	Minimum-maximum	*p*-Value
CEA, μg/l	Healthy	2.33	1.60	1.10	2.50	0.30-11.00	<0.0001
	Cancer	8.01	2.80	1.70	4.90	0.40-426.00	
CYFRA 21-1, μg/l	Healthy	1.77	1.40	1.10	2.10	0.50-6.40	<0.0001
	Cancer	4.89	2.20	1.50	3.50	0.60-81.90	
NSE, μg/l	Healthy	12.18	11.60	10.05	13.40	6.20-32.80	0.0447
	Cancer	13.61	12.50	10.60	15.00	6.20-34.20	
TPS, IU/l	Healthy	74.31	52.00	31.00	83.00	10.00-892.00	0.0755
	Cancer	90.76	59.00	37.00	98.00	10.00-727.00	
MonoTotal, IU/l	Healthy	123.95	92.00	65.50	131.90	15.00-904.60	0.0065
	Cancer	160.86	124.20	76.50	176.60	15.00-1034.20	
IGF-1, μg/l	Healthy	129.8	128.00	89.00	162.00	35.00-281.00	0.0020
	Cancer	151.76	146.00	115.00	183.00	57.00-332.00	
Pro-GRP, ng/l	Healthy	42.04	36.00	28.00	52.50	13.00-176.00	0.0232
	Cancer	46.21	42.00	33.00	51.00	17.00-163.00	

CEA: Carcinoembryonic antigen; CYFRA 21-1: cytokeratin-19 fragments; IGF-1: insulin-like growth factor 1; MonoTotal: soluble fragments of cytokeratin 8, 18 and 19; NSE: neuron-specific enolase; Pro-GRP: pro-gastrin-releasing peptide; TPS: tissue polypeptide-specific antigen.

(p=0.0447), MonoTotal (p=0.0065), IGF-1 (p=0.0020), and pro-GRP (p=0.0232).

ROC curves were plotted and areas under the curve (AUC) were calculated for individual tumor markers. A model of three markers, namely CYFRA 21-1, CEA and IGF-1, was created using a multivariate analysis. The AUC of this model was higher than the AUC values of individual tumor markers. The AUC value decreased as follows: 3-Marker model > CYFRA 21-1 > CEA > IGF-1 > MonoTotal > NSE > proGRP >TPS.

The AUC values are given in Table III, the ROC curve of the multivariate model is shown in Figure 1.

To determine the distribution of tumor markers according to the tumor size, patient results were divided into groups according to the pT classification described in the eighth edition of the TNM Classification of Malignant Tumours (8). Of the tumor markers selected, levels of CYFRA 21-1 and MonoTotal statistically significantly increased with the size of the tumor (p=0.0002 and p=0.0236, respectively). The results are shown in Table IV.

In our study, no relationship was observed between the levels of tumor markers and lymph node status (pN).

As can be seen from Table V, the levels of CYFRA 21-1 and MonoTotal significantly increased with the stage of NSCLC (p<0.0001 and p=0.0063, respectively).

Different levels of tumor markers were observed in tumors that differed histologically. Epidermoid lung carcinoma was associated with the highest level of CYFRA 21-1 (mean=9.00 μg/l, p<0.0001). Adenocarcinoma was associated with the highest level of CEA (mean=12.58 μg/l, p=0.0213).

Follow-up after surgery revealed that 26 patients experienced tumor recurrence despite surgery. Tumor recurrence was associated with a statistically significant increase in CEA and CYFRA 21-1 (p=0.0082 and p=0.0453, respectively) prior to recurrence.

Table III. *The area under the receiver operating characteristics curve (AUC) for individual markers and those included in the multivariate model, namely carcinoembryonic antigen (CEA), cytokeratin-19 fragments (CYFRA 21-1) and insulin-like growth factor 1 (IGF-1).*

Order	Tumor marker	AUC value
1	CYFRA 21-1	0.6882
2	CEA	0.6857
3	IGF-1	0.6225
4	MonoTotal	0.6078
5	Pro-GRP	0.5981
6	NSE	0.5835
7	TPS	0.5700
-	Multivariate model	0.7730

MonoTotal: Soluble fragments of cytokeratin 8, 18 and 19; NSE: neuron-specific enolase; Pro-GRP: pro-gastrin-releasing peptide; TPS: tissue polypeptide-specific antigen.

Discussion

The detection and measurement of serum tumor markers as part of the treatment of malignant lung tumors, especially as part of a follow-up, is a topic that has long been discussed in the literature (9). The unsatisfactory 5-year survival and poor prognosis of a relatively high percentage of patients is in itself sufficient justification for further research into tumor markers; honing the methodology of their correct use is imperative (10).

The panel of tumor markers in this study was selected based on our own experience in combination with the

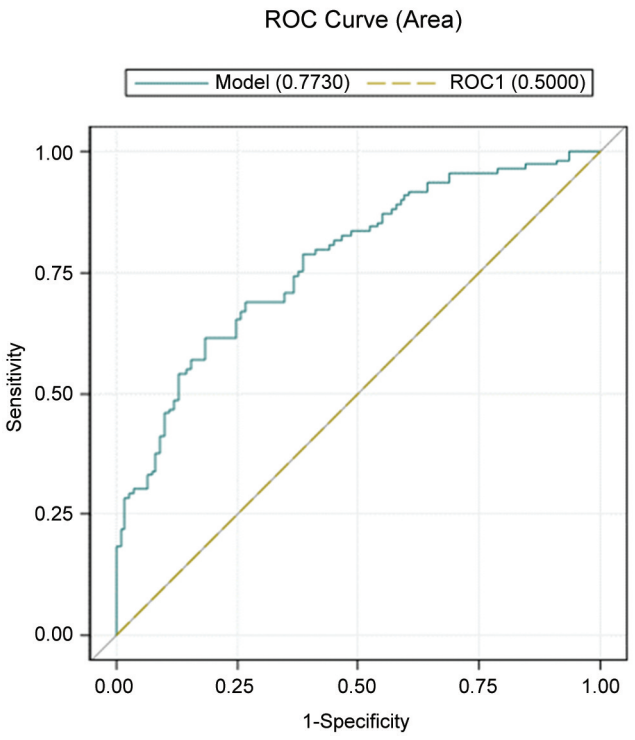

Figure 1. *Receiver operating characteristic curve – multivariate model.*

Table IV. *Serum levels (mean±SD) of cytokeratin-19 fragments (CYFRA 21-1) and soluble fragments of cytokeratins 8, 18 and 19 (MonoTotal) according to the extent of primary tumor (pT).*

pT	Frequency	CYFRA 21-1, μg/l	MonoTotal, IU/l
T1mi	1	1.20	76.5
T1a	5	1.70±0.51	95.4±39.36
T1b	32	1.79±0.72	123±85.34
T1c	22	2.17±0.88	118±57.63
T2a	22	6.54±16.95	141±123.22
T2b	8	4.93±3.05	272±275.72
T3	10	9.59±7.96	188±116.18
T4	9	15.5±18.68	362±310.27
Total	109	-	-
p-Value	-	0.0002	0.0236

Table V. *Serum levels (mean±SD) of cytokeratin-19 fragments (CYFRA 21-1) and soluble fragments of cytokeratins 8, 18 and 19 (MonoTotal) according to tumor stage.*

Stage	Frequency	CYFRA 21-1, μg/l	MonoTotal, IU/l
I	64	2.12±1.31	116±70.44
II	24	9,088.50±16.62	219±196.33
III	21	8,589.08±13.55	231±237.04
Total	109	-	-
p-Value	-	<0.0001	0.0063

literature findings. Unlike in the literature, where it is most common to evaluate the benefit of one or two tumor markers, our study evaluated a number of individual markers and a multivariate analysis was carried out in order to determine the most effective combination of tumor markers.

The preoperative levels of all selected tumor markers except TPS were statistically significantly higher in patients with NSCLC than in persons from the control group. This corresponds to the findings described in the literature (11-16). To evaluate in detail the contribution of individual markers, ROC curves were generated and the AUCs calculated. The two best-performing tumor markers were then selected for the multivariate model using a multivariate analysis (CYFRA 21-1 and CEA) and a third marker (IGF-1), the course of which did not correlate with any other marker used, was added. The AUC for the generated model (0.7730) was statistically significantly higher (p=0.0050) than the AUC of the best individual tumor marker (CYFRA 21-1). The use of such a model has not been described in the literature to date as far as we are aware.

The correlation between the concentration of individual tumor markers and the size and stage of the tumor was measured. Our data did not confirm the commonly mentioned assumption that CEA is a tumor marker that exhibits a rise as the mass of the tumor increases (16). It did however reflect a statistically significant correlation between tumor size and the concentration of two cytokeratin markers: CYFRA 21-1 (p=0.0002) and MonoTotal (p=0.0236). Our results are consistent with the results of studies reported in available publications (9, 12, 13, 17-19). According to the published studies, the levels of CYFRA 21-1 and MonoTotal depend on the clinical stage (9, 12, 13, 18). Our study confirms this. Both these cytokeratins statistically significantly increased with increasing stage of the disease: CYFRA 21-1 (p<0.0001) and MonoTotal (p=0.0063). In contrast to the literature findings, we did not find any relation between the concentration of the studied tumor markers and the degree of nodal involvement.

The levels of tumor markers varied according to the histological type of the tumor. In our study, epidermoid lung carcinoma was associated with the highest level of CYFRA 21-1 and adenocarcinoma was associated with the highest level of CEA, in concordance with the literature (9, 20, 21).

The final aspect of tumor markers to be addressed in our study was their individual prognostic ability. A Cox regression model with time-dependent covariates (individual tumor markers) was used to assess DFI. Recurrence was associated with a statistically significant increase in CEA and CYFRA 21-1 (p=0.0082 and p=0.0453, respectively) prior to recurrence, in line with literature data (12, 22-24).

In conclusion, our study shaved that CEA and CYFRA 21-1 play a key role in the detection of lung cancer recurrence and its prognosis. Other tumor markers we selected did not provide additional information when used as individual markers. A multivariate approach can be used to increase the effectiveness of the detection of recurrence.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization: JV and RK; methodology: JV, RK, JF and OT; investigation: JS, MS, KP, MS and BV; statistical analyses: LP; writing – original draft preparation: JV and RK; writing – review and editing: JV, RK, OT, VT and MB.

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References

1 Barta JA, Powell CA and Wisnivesky JP: Global epidemiology of lung cancer. Ann Glob Health *85(1)*: 8, 2019. PMID: 30741509. DOI: 10.5334/aogh.2419

2 Neal RD, Sun F, Emery JD and Callister ME: Lung cancer. BMJ *365*: l1725, 2019. PMID: 31160279. DOI: 10.1136/bmj.l1725

3 Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, Bonaventure A, Valkov M, Johnson CJ, Estève J, Ogunbiyi OJ, Azevedo E Silva G, Chen WQ, Eser S, Engholm G, Stiller CA, Monnereau A, Woods RR, Visser O, Lim GH, Aitken J, Weir HK, Coleman MP and CONCORD Working Group: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet *391(10125)*: 1023-1075, 2018. PMID: 29395269. DOI: 10.1016/S0140-6736(17)33326-3

4 Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L: Lung cancer: current therapies and new targeted treatments. Lancet *389(10066)*: 299-311, 2017. PMID: 27574741. DOI: 10.1016/S0140-6736(16)30958-8

5 Yang RH, Tian RF, Ren QL, Chui HY, Guo ST, Zhang XD and Song X: Serum protein profiles of patients with lung cancer of different histological types. Asia Pac J Clin Oncol *12(1)*: 70-76, 2016. PMID: 26668125. DOI: 10.1111/ajco.12441

6 Boyer M, Tsao MS, Jänne P, Ramalingam S, Pitman Lowenthal S and Alam M: Preparing for tomorrow: molecular diagnostics and the changing nonsmall cell lung cancer landscape. Asia Pac J Clin Oncol *10 Suppl 2*: 2-10, 2014. PMID: 24673735. DOI: 10.1111/ajco.12189

7 Rusch VW, Asamura H, Watanabe H, Giroux DJ, Rami-Porta R, Goldstraw P and Members of IASLC Staging Committee: The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol *4(5)*: 568-577, 2009. PMID: 19357537. DOI: 10.1097/JTO.0b013e3181a0d82e

8 Bertero L, Massa F, Metovic J, Zanetti R, Castellano I, Ricardi U, Papotti M and Cassoni P: Eighth Edition of the UICC Classification of Malignant Tumours: an overview of the changes in the pathological TNM classification criteria-What has changed and why? Virchows Arch *472(4)*: 519-531, 2018. PMID: 29209757. DOI: 10.1007/s00428-017-2276-y

9 Molina R, Agusti C, Filella X, Jo J, Joseph J, Giménez N and Ballesta AM: Study of a new tumor marker, CYFRA 21-1, in malignant and nonmalignant diseases. Tumour Biol *15(6)*: 318-325, 1994. PMID: 7997803. DOI: 10.1159/000217908

10 Duffy MJ and O'Byrne K: Tissue and blood biomarkers in lung cancer: a review. Adv Clin Chem *86*: 1-21, 2018. PMID: 30144837. DOI: 10.1016/bs.acc.2018.05.001

11 Xu CM, Luo YL, Li S, Li ZX, Jiang L, Zhang GX, Owusu L and Chen HL: Multifunctional neuron-specific enolase: its role in lung diseases. Biosci Rep *39(11)*: BSR20192732, 2019. PMID: 31642468. DOI: 10.1042/BSR20192732

12 He Y, Cui Y, Chang D and Wang T: Postoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I pulmonary adenocarcinoma. Oncotarget *8(42)*: 73115-73122, 2017. PMID: 29069854. DOI: 10.18632/oncotarget.17611

13 Prazakova M, Vrzalova J, Auge JM, Safranek J, Topolcan O, Fuchsova R, Spisakova M, Svobodova S, Holubec L Jr and Pesta M: The role of MonoTotal in the primary diagnosis, prognosis and follow-up of patients with non-small cell lung cancer (NSCLC). Anticancer Res *31(9)*: 3107-3112, 2011. PMID: 21868567.

14 Patel O, Shulkes A and Baldwin GS: Gastrin-releasing peptide and cancer. Biochim Biophys Acta *1766(1)*: 23-41, 2006. PMID: 16490321. DOI: 10.1016/j.bbcan.2006.01.003

15 Yu H, Spitz MR, Mistry J, Gu J, Hong WK and Wu X: Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. J Natl Cancer Inst *91(2)*: 151-156, 1999. PMID: 9923856. DOI: 10.1093/jnci/91.2.151

16 Zhang M, Li X, Zhang X, Yang Y, Feng Z and Liu X: Association of serum hemoglobin A1c, C-peptide and insulin-like growth factor-1 levels with the occurrence and development of lung cancer. Mol Clin Oncol *2(4)*: 506-508, 2014. PMID: 24940485. DOI: 10.3892/mco.2014.289

17 Treska V, Topolcan O, Zoubkova V, Treskova I, Narsanska A and Kucera R: Perioperative tumour marker levels as prognostic factors for surgical treatment of breast cancer liver metastases. Anticancer Res *38(6)*: 3647-3652, 2018. PMID: 29848722. DOI: 10.21873/anticanres.12640

18 Bates J, Rutherford R, Divilly M, Finn J, Grimes H, O'Muircheartaigh I and Gilmartin JJ: Clinical value of CYFRA 21.1, carcinoembryonic antigen, neurone-specific enolase, tissue polypeptide specific antigen and tissue polypeptide antigen in the diagnosis of lung cancer. Eur Respir J *10(11)*: 2535-2538, 1997. PMID: 9426091. DOI: 10.1183/09031936.97.10112535

19 Kornek G, Schenk T, Raderer M, Djavarnmad M and Scheithauer W: Tissue polypeptide-specific antigen (TPS) in monitoring palliative treatment response of patients with

gastrointestinal tumours. Br J Cancer *71(1)*: 182-185, 1995. PMID: 7529527. DOI: 10.1038/bjc.1995.37

20 Watanabe T, Sawabata N, Kawaguchi T, Yoshikawa D, Kushibe K, Nakane S and Taniguchi S: Clustered circulating tumor cells in lung adenocarcinoma: implications of test results of continuous variables. Anticancer Res *40(12)*: 7089-7094, 2020. PMID: 33288607. DOI: 10.21873/anticanres.14737

21 Zhou J, Diao X, Wang S and Yao Y: Diagnosis value of combined detection of serum SF, CEA and CRP in non-small cell lung cancer. Cancer Manag Res *12*: 8813-8819, 2020. PMID: 33061577. DOI: 10.2147/CMAR.S268565

22 Nasralla A, Lee J, Dang J and Turner S: Elevated preoperative CEA is associated with subclinical nodal involvement and worse survival in stage I non-small cell lung cancer: a systematic review and meta-analysis. J Cardiothorac Surg *15(1)*: 318, 2020. PMID: 33059696. DOI: 10.1186/s13019-020-01353-2

23 Zhai Y, Hui Z, Men Y, Luo Y, Gao Y, Kang J, Sun X and Wang J: Combined neat model for the prognosis of postoperative stage III-N2 non-small cell lung cancer. Thorac Cancer *11(9)*: 2610-2617, 2020. PMID: 32725794. DOI: 10.1111/1759-7714.13585

24 Liu C, Huang Q, Ma W, Qi L, Wang Y, Qu T, Sun L, Sun B, Meng B and Cao W: A combination of tumor and molecular markers predicts a poor prognosis in lung adenocarcinoma. Int J Clin Exp Pathol *12(5)*: 1690-1701, 2019. PMID: 31933987.

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