Med Oncol (2013) 30:551
DOI 10.1007/s12032-013-0551-6

ORIGINAL PAPER

The prognostic importance of changing serum M30 and M65 values after chemotherapy in patients with advanced-stage non-small-cell lung cancer

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Received: 11 February 2013 / Accepted: 20 March 2013 / Published online: 28 March 2013

Abstract Although oncological treatments are improving, the prognosis of non-small-cell lung cancer (NSCLC) patients has not. Several biomarkers related to prognosis have been evaluated, and M30 and M65 have been reported to be higher in patients with NSCLC than in healthy people. In the current study, we evaluated the clinical importance of the change in serum M30 and M65 values after chemotherapy in patients with NSCLC. Serum M30 and M65 values were measured before and 48 h after chemotherapy in thirty-two patients with advanced NSCLC. The importance of the change in the levels of these markers after chemotherapy was analyzed by univariate analysis. The median serum M65 and M30 values increased significantly after chemotherapy ($p < 0.001$). The median M30 value after chemotherapy was an important prognostic factor for both overall survival (OS) ($p = 0.002$) and progression-free survival (PFS) ($p = 0.002$). Stage and histopathological type were significant both for PFS and OS. Multivariate analysis showed that the median M30 value after chemotherapy was the only independent prognostic factor for PFS ($p = 0.04$, HR 5.4) and OS ($p = 0.02$, HR 11.49). Our results indicated that both serum M30 and M65 values increased after chemotherapy in patients with advanced NSCLC, and an elevated serum M30 value was an independent prognostic factor for both PFS and OS.

Keywords M30 · M65 · Lung cancer · Prognosis

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Introduction

Cytokeratin 18 (CK-18) is an intermediate filament that is found in epithelial cells and is released into the circulation during cell death [1, 2]. During apoptosis, CK-18 is cleaved by caspases into a proteolytic component [3]. M30 is the caspase-cleaved derivative of CK-18, and M65 is the intact form of CK-18; they were both detected in the circulation by enzyme-linked immunosorbent assays (ELISA) [4]. M30 is a monoclonal antibody that recognizes the only caspase-cleaved neoepitope of CK-18 during apoptosis. The monoclonal antibody M65 detects all CK-18 fragments that contain full-length epitopes of the protein, which are released during both apoptotic and necrotic cell death. [3]. Most chemotherapeutic agents act by inducing apoptosis, which increases significantly 24 h following chemotherapy [5]. Apoptosis can be measured in the serum by several biomarkers to predict the efficacy of drugs [6, 7]. In two studies, Kramer et al. [8] and De Haas et al. [9] reported an increase in serum M30 levels after chemotherapy in prostate and testis cancer patients. In addition, Olofsson et al. [10] and de Haas et al. [9] reported that serum M65 levels were elevated after chemotherapy, which implies that M65 is important for predicting chemotherapy-induced cell death.

Increased levels of circulating CK-18 fragments have been reported as important predictors of prognosis or treatment response in several solid tumors [1, 3, 9, 11, 12].

An increased M65 level was reported as a poor prognostic indicator for pancreatic [13] and testicular cancer [9], and an increased M30 level was related to survival in gastric [1] and testicular cancer [9].

Lung cancer is the leading cause of cancer-related mortality worldwide. The 5-year overall survival (OS) rate for NSCLC is approximately 15 % [14]. Recognition of new biomarkers that can be used to evaluate prognosis may improve the outcome of patients with lung cancer. Ulukaya et al. [15] first reported that M30 levels were higher in lung cancer patients than in patients with benign lung disease or healthy individuals. They reported that the basal serum M30 level was associated with prognosis and that after chemotherapy, serum M30 values were increased fourfold. There is only one prior study related to the importance of the change in serum M30 levels after chemotherapy in lung cancer patients and none for serum M65 levels. It is desirable to assess the amount of cell death induced by chemotherapy for the management of patients and the determination of treatment response. M30 and M65 were suitable, noninvasive markers for apoptosis and cell death [8]. In this study, we aimed to evaluate the prognostic importance of the change in the M30 and M65 serum levels after chemotherapy in advanced NSCLC patients.

Materials and methods

The study included 32 patients with advanced-stage NSCLC who were treated consecutively and received follow-up care at the Dr. Lutfi Kirdar Research and Education Hospital, Department of Medical Oncology, between 2008 and 2010. Bronchoscopy or operation was performed for the histopathological confirmation for all patients, and patients were staged as locally advanced or metastatic according to the American Joint Committee on Cancer (AJCC), 7th version [16]. All patients were treated with the platinum–taxane combination (cisplatine 75 mg/m^2–dosetaxel 75 mg/m^2, ciaplatin–paclitaxel 175 mg/m^2, carboplatine AUC5–paclitaxel 175 mg/m^2, carboplatine AUC5–docetaxel 75 mg/m^2, 1/21 days of cycle).

Patient selection

Patients were included in the study if they were chemotherapy-naive or had not received chemotherapy in the 6 months prior to the study; they were also required to have an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$2 with normal levels of renal function and hepatic enzymes and adequate bone marrow (WBC count $\geq$3,000/mm^3, platelet count $\geq$100,000/mm^3). Patients with secondary malignancies, except for basal-cell carcinoma of the skin or cervical carcinoma in situ, were excluded. Approval from the Local Ethics Committee of our hospital was achieved before beginning the study. Clinical information, such as age, gender, performance status, operations performed, tumor stage, and histopathological type, was obtained from the patients' medical records after informed consent was received from the patients or their relatives.

Serum samples

Five milliliter blood samples were collected from the peripheral blood vessels of 32 patients into dry tubes before and 48 h after chemotherapy administration. The samples were centrifuged at 1,000*g* for 10 min and stored at −20 °C until evaluation 4 months later. All samples were analyzed simultaneously.

M30 and M65 measurement

The serum M30 and M65 values were measured using M30-Apoptosense and M65 ELISA kits obtained from Peviva AB (Sweden) according to manufacturer's instructions. The ELISA used a monoclonal antibody for recognition of CK-18 and horseradish peroxidase conjugated to M30 for detection. Excess unbound conjugate was removed, and then, TMB substrate was added. Finally, absorbance was measured in a microplate reader at 450 nm. By plotting a standard curve from known concentrations measured absorbances, M30 and M65 levels were determined and expressed in U/L. Units of M30 and M65 ELISA were defined using a synthetic peptide (1 U = 1.24 pmol). The measuring range for M30 and M65 levels was 0–1,000 and 0–2,000 U/L, respectively.

The measured serum M30 and M65 levels before and after chemotherapy were compared.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. The median serum M30 and M65 values which were calculated before and after chemotherapy were compared using the Wilcoxon test. The relationship between median serum M30 and M65 values after chemotherapy and other clinicopathological factors were compared by the chi-squared and Fisher's exact tests. Survival analysis and curves were established according to the Kaplan–Meier method and compared by the log-rank test. OS was measured from the time of initial diagnosis until the date of the patient's death or the termination of patient follow-up. Progression-free survival (PFS) was defined as the time from diagnosis until disease progression, the date of death, or termination of patient follow-up. Univariate analyses were carried out to evaluate the important prognostic factors. Then, multivariate analysis with the Cox proportional hazards model was performed to

further analyze independent prognostic factors that were found in the univariate analysis to predict OS and PFS. The response to chemotherapy was evaluated according to WHO criteria at 3 months after chemotherapy and classified as progressive disease (PD), stable disease, or partial response (PR) [17]. The logistic regression analysis was performed to evaluate the relationship between treatment response and elevated serum M30 and M65 levels after chemotherapy. The 95 % confidence interval (CI) was used to quantitate the relationship between survival time and each independent factor. All p values were two-sided in the tests, and p values equal to or less than 0.05 were considered to be statistically significant.

Results

Patient characteristics

A total of 32 patients with advanced-stage NSCLC were included in the study. The median age of the patients was 59 years (range 44–72), and 94 % of them were male. Half of the patients were locally advanced and staged as IIIA ($n = 3$) or IIIB ($n = 13$), and the remaining patients were stage IV. The most frequent site of metastasis was bone (62 %), but 31 % of the patients had multiple metastases. While histopathological subtypes could not be determined in 15.6 % of the patients, 62.5 % of the cancers were classified as squamous-cell carcinomas (SCC), and 21.9 % were classified as adenocarcinomas.

Serum M30 and M65 values

The median serum M30 and M65 values were 275.5 and 992.5 U/L, respectively, before chemotherapy. On the other hand, after chemotherapy, the median values were 550.2 and 1458.9 U/L, respectively. 48 h after chemotherapy, both serum M30 and M65 values increased significantly ($p < 0.001$ for both, Table 1). In patients with metastatic disease, serum M65 values were significantly higher after chemotherapy than in patients with locally advanced disease ($p = 0.001$). In addition, the patients who had undergone an operation had lower serum M65 values after chemotherapy than patients who had not undergone an operation ($p = 0.01$). Although patients with metastatic disease had higher M30 values after chemotherapy compared to patients without metastasis, the difference was not statistically significant ($p = 0.07$). There was no relationship between serum M30 and M65 values and the other clinicopathological factors, such as age, gender, or histopathological type (Table 2).

Overall survival and progression-free survival

The median OS and PFS times were 18.2 and 11.9 months, respectively, with a median follow-up period of 13.3 months (range 1.6–73). In addition, the 2 year OS and PFS rates were 39.9 and 26 %, respectively.

Prognostic and predictive importance of serum M30 and M65 values

The histopathological type, stage, and serum M30 values after chemotherapy were found to be important prognostic factors for both PFS and OS by univariate analysis. The median PFS and OS times were longer for NSCLC compared to other subtypes ($p = 0.01$ and $p = 0.02$, respectively). The median PFS times were 29.8 months for NSCLC, 17.8 months for adenocarcinomas, and 11.1 months for SCC. Moreover, the patients with locally advanced-stage NSCLC had longer PFS and OS than metastatic patients ($p = 0.01$ and $p = 0.008$, respectively). Patients with serum M30 values $\leq$550.2 U/L after chemotherapy had longer PFS (17 vs. 6.9 months, $p = 0.002$) and OS ($p = 0.002$) than patients with serum M30 values >550.2 U/L. Although patients with serum M65 values $\leq$1458.9 U/L after chemotherapy had longer PFS than patients with serum M65 values >1458.9 U/L, this difference was not significant (17.8 vs. 11.1 months, $p = 0.08$). There was no correlation between PFS and OS and other clinical factors, such as age, gender, the presence of operation, or serum M30 and M65 values before chemotherapy ($p > 0.05$). The results of the univariate analysis are shown in Table 3. OS and PFS curves of patients according to serum M30 values after chemotherapy are shown in Figs. 1 and 2. By logistic regression, there was no relation between treatment response and the median post-chemotherapy serum values for both M30 and M65 ($p = 0.3$ vs. $p = 0.5$).

The serum M30 value after chemotherapy was an independent prognostic factor for both OS ($p = 0.02$, HR 11.5; 95 % CI 1.3–100.3) and PFS ($p = 0.04$, HR 5.8; 95 % CI 1–32.5) by multivariate analysis. The results of multivariate analysis are shown in Tables 4 and 5.

Discussion

The main mechanism of chemotherapy is killing cancer cells by the induction of apoptosis; therefore, in vitro analysis of apoptotic biomarkers has been used to test the efficacy of chemotherapy drugs [18]. Serum levels of different forms of CK-18 reflect the type of cell death occurring in the body [2]. M30 is a caspase-cleaved form of CK-18 that is released into the extracellular space following breakdown of the cell membrane during apoptosis of

Table 1 The changing serum M30 and M65 values after the chemotherapy

	Mean serum value before the CT (U/L)	Range	Mean serum value after the CTCT (U/L)	Range	*p*
M30 (U/L)	275.5	144–2970	550.2	214–6000	<0.001
M65(U/L)	992.5	409–9438	1458.9	535–6292	<0.001

CT chemotherapy

Table 2 The relationship between clinicopathological characteristics and the difference of serum median M30 and M65 values after chemotherapy

Variables	Median M30 (U/L) after CT			Median M65 (U/L) after CT		
	>550.2	≤550.2	*p*	>1458.9	≤1458.9	*p*
Gender			1			1
Female	1 (6.2)	1 (6.2)		1 (6.2)	1 (6.2)	
Male	15 (93.8)	15 (93.8)		15 (93.8)	15 (93.8)	
Age			1			1
>55	12 (75)	12 (75)		12 (75)	12 (75)	
≤55	4 (25)	4 (25)		4 (25)	4 (25)	
Stage			0.07			0.001
Metastatic	11 (68.7)	5 (31.3)		13 (81.2)	3 (18.8)	
Locally advanced	5 (31.3)	11 (68.7)		3 (18.8)	13 (81.2)	
Operation			0.3			0.01
Present	1 (6.2)	4 (25)		0 (0)	5 (31.2)	
Absent	15 (93.8)	12 (75)		16 (100)	11 (68.8)	
Histopathological type			0.2			0.2
Adenocarcinoma	3 (18.7)	4 (25)		3 (18.7)	4 (25)	
SCC	12 (75)	8 (50)		12 (75)	8 (75)	
NSCLC	1 (6.3)	4 (25)		1 (6.3)	4 (25)	

epithelial cells. M65 indicates the total amount of CK18 fragments released during tissue necrosis in addition to caspase-cleaved fragments of CK-18 [1, 4].

In the present study, we found that both serum M30 and M65 levels were significantly increased 48 h after chemotherapy in NSCLC patients. Elevated serum M65 levels were significantly correlated with disease stage. The occurrence of an operation and an increased level of M30 post-chemotherapy were predictors of survival. Patients with serum M30 values >550.2 U/L had shorter OS and PFS than patients with serum M30 ≤550.2 U/L. M30 and M65 levels have been previously reported to increase within 1–3 days after chemotherapy in prostate and breast cancer patients [8, 10]. Kramer et al. [8] reported that serum M30 and M65 levels were elevated at 1, 3, 5, and 7 days after docetaxel chemotherapy in advanced prostate cancer patients. Demiray et al. [7] evaluated the serum M30 level before and 24 and 48 h after chemotherapy in 42 patients with breast cancer. They reported that the serum M30 level increased significantly 24 and 48 h post-chemotherapy and that this change was a predictor of the tumor response. Similarly, Ulukaya et al. [6] found that serum M30 levels increased in breast cancer patients 24 and 48 h after neoadjuvant chemotherapy. In their study, the increase in serum M30 levels was related to treatment response; the treatment responsive group had higher serum M30 values compared to the nonresponsive groups. Compatible with previous studies, we also measured serum M30 and M65 levels 48 h after chemotherapy, but in our study, post-chemotherapy values did not predict treatment response. If we follow patients for a longer time, we may find a correlation. We only confirmed the prognostic importance of post-chemotherapy M30 levels in NSCLC patients. In 11 testicular cancer patients in the de Haas' study, serum M30 and M65 levels were elevated significantly at 1, 2, 5, and 7 days after chemotherapy and then decreased to a baseline level after 14 days [9]. The decrease in these levels after 14 days reflected the decrease in tumor load as a result of chemotherapy-induced cell death [9]. In the current study, we did not measure M30 and M65 serum levels before the start of the 2nd cycle of chemotherapy, so we could not determine the change in M30 and M65 levels after the 2nd cycle of chemotherapy.

Yaman et al. [1] showed that serum M30 and M65 levels were significantly higher in advanced gastric cancer patients than in a healthy control group. In addition, patients with metastatic disease had higher serum M30 levels than the locally advanced group. While M30 levels were associated with poor prognosis, M65 levels were not. Ueno et al. [3] showed that serum CK-18 levels were higher in breast cancer

Table 3 The results of univariate analysis for overall survival and progression-free survival

Characteristics	Number (%)	Median PFS (range)	*p*	Median OS (range)	*p*
Age			0.8		0.5
>50	24 (75)	11.9 (9.2–14.6)		18 (14.4–21.6)	
≤50	8 (25)	13.4 (6.1–20.7)		na	
Gender			0.7		na
Male	30 (93.8)	na		na	
Female	2 (6.3)				
Operation			0.1		0.2
Present	5 (15.6)	27.5 (1.1–53.8)		na	
Absent	27 (84.4)	11.9 (9.5–14.3)		15.8 (6.7–25.4)	
Histopathological type			0.01		0.02
Adenocarcinoma	7 (21.9)	17.8 (16.2–19.4)		na	
SCC	20 (62.5)	11.1 (6.2–15.9)		15.8 (6.7–25.4)	
NSCLC	5 (15.6)	29.8 (nr)		na	
Stage			0.01		0.008
Locally advanced	16 (50)	17.8 (2.3–33.2)		13.4 (6.9–20)	
Metastatic	16 (50)	11.1 (4.2–17.9)		na	
Median M30 (U/L) before CT			0.1		0.9
>275.5	16 (50)	11.9 (9.9–14)		18.6 (12.1–25.1)	
≤275.5	16 (50)	13.6 (0–31.3)		18 (14.8–21.1)	
Median M65 (U/L) before CT			0.2		0.6
>992.5	16 (50)	11.9 (9.9–14)		18.2 (10.2–26.1)	
≤992.5	16 (50)	13.4 (10.1–16.7)		18 (14.5–21.4)	
Median M30 (U/L) after CT			0.002		0.002
>550.2	16 (50)	6.9 (6.2–7.7)		7.8 (2.2–13.4)	
≤550.2	16 (50)	17 (2–32)		na	
Median M65 (U/L) after CT			0.08		0.06
>1458.9	16 (50)	11.1 (4.1–18)		15.8 (5.5–26.2)	
≤1458.9	16 (50)	17.8 (8.5–27)		na	

Na not available

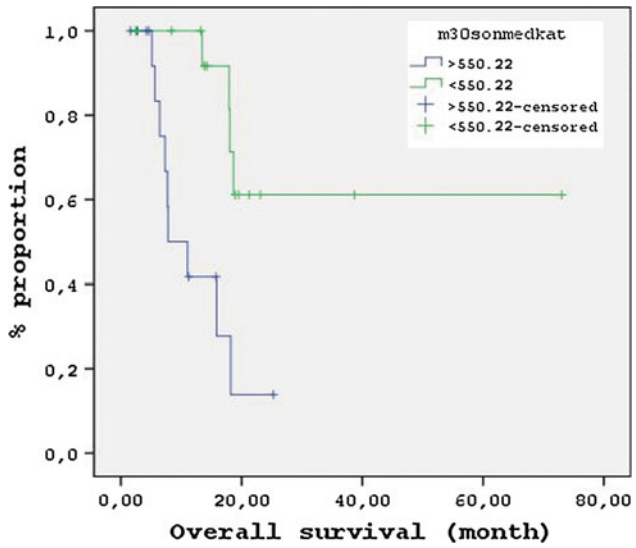

Fig. 1 The overall survival curve according to the median serum M30 levels after chemotherapy

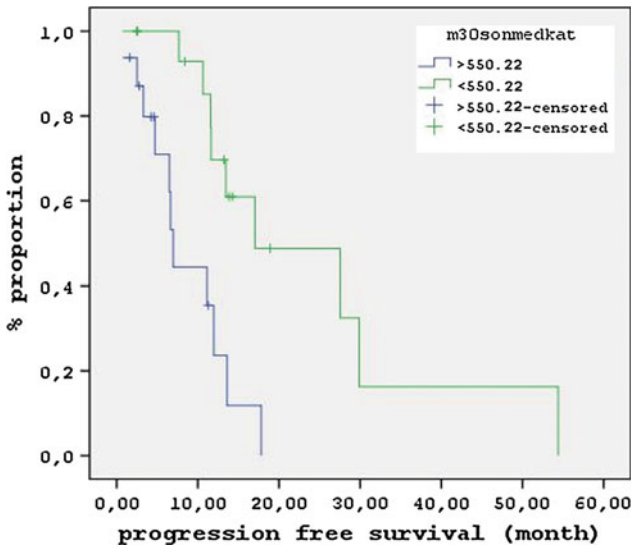

Fig. 2 The progression-free survival curve according to the median serum M30 levels after chemotherapy

Table 4 The results of multivariate analysis for overall survival

Characteristics	Wald	*p*	95 %CI	HR
Stage	2.5	0.1	0.6–55.4	6.07
Histopathology	2.9	0.08	0.7–116.4	9.16
M30 level (U/L) after CT	4.8	0.02	1.3–100.3	11.49
M65 level (U/L) after CT	2.2	0.1	0.009–1.9	0.13

Table 5 The results of multivariate analysis for progression-free survival

Characteristics	Wald	*p*	95 %CI	HR
Stage	2.1	0.1	0.6–18.1	3.48
Histopathology	3.6	0.05	0.9–99.7	9.68
Operation	0.7	0.3	0.05–3	0.41
M30 level (U/L) after CT	3.9	0.04	1–32.5	5.77
M65 level (U/L) after CT	2.1	0.1	0.03–1.6	0.22

patients who received chemotherapy; however, they could not find any relationship between M30 levels and prognosis [3]. Similarly, we previously reported that serum M30 levels were elevated in gastric cancer patients with metastatic disease compared to the locally advanced group. Both elevated plasma M30 and M65 levels were associated with poor prognosis with respect to PFS, but this significance could not be confirmed by multivariate analysis [11]. Our study is the first to show the association of post-chemotherapy serum M30 values with poor prognosis in lung cancer, but baseline values of these biomarkers were not related to prognosis. Koelink et al. evaluated plasma M30 and total CK18 levels in 49 colorectal cancer patients before and after surgical resection. They reported that M30 and M65 levels were significantly correlated with disease-free survival (DFS) before and after resection. While the serum M30 level was an independent prognostic factor for DFS by multivariate analysis, M65 was not. They reported that both of these biomarkers were related with disease stage, tumor diameter, and DFS [12]. Dive et al. [13] reported higher median M65 levels in metastatic pancreatic cancer patients than in patients with locally advanced or resectable pancreatic cancer. In addition, they found that M65 levels were associated with poor OS in a univariate analysis, but they could not confirm this with a multivariate analysis. In our study, we also found that patients with metastatic disease had a higher post-chemotherapy serum M65 level than patients with locally advanced disease. Moreover, metastatic patients had higher serum M30 levels after chemotherapy, but this was not statistically significant ($p = 0.07$). We also found that the serum M30 level after chemotherapy was an independent prognostic factor for both OS and PFS in advanced-stage NSCLC.

Hou et al. [19] documented that serum M30 and M65 levels and the number of circulating tumor cells were higher and served as important prognostic factors for survival in small cell lung cancer (SCLC) patients. The prognostic significance of serum M30 levels, but not serum M65 levels, was studied firstly in NSCLC patients by Ulukaya et al. [15]. They observed evaluated serum M30 levels in 18 lung cancer patients before and 24 and 48 h after chemotherapy. Both NSCLC and small cell lung carcinoma (SCLC) patients were included in their study. This was different from our study, which included 32 patients who all had NSCLC. Ulukaya et al. reported a fourfold increase in serum M30 levels after chemotherapy, but they did not evaluate serum M65 levels in their study. They indicated that there was no difference in the serum value of M30 24 or 48 h after chemotherapy, so we only measured the serum M30 level 48 h after chemotherapy. Ulukaya et al. reported that basal serum M30 values were a poor prognostic factor for survival. We did not find any relationship between basal serum M30 or M65 levels and survival, but post-chemotherapy elevated median serum M30 values were a poor prognostic factor for both OS and PFS. This was different from previous findings. Moreover, by multivariate analysis, it was an independent prognostic factor for survival. In NSCLC patients, we demonstrated that an elevated serum M30 level after chemotherapy indicated that apoptotic cell death had been induced by chemotherapy and, therefore, may be used to predict early prognosis and make an appropriate treatment plan.

De Haas et al. reported that pre-chemotherapy serum M30 and M65 values were correlated with LDH, AFP, and β-hcG in testicular cancer. Moreover, serum M30 and M65 levels were correlated with the International Germ Cell Consensus (IGCC) prognostic group. Both of these markers were highest in poor prognostic groups [9]. In another study, serum M30 levels were correlated with grade, stage, and Ki-67 index in patients with endometrial cancer [20]. In contrast to previous studies, we analyzed the relationship between post-chemotherapy serum M30 and M65 levels and other clinical factors. We found that only serum M65 values were associated with the stage of disease and the occurrence of an operation. There was no relationship between the M30 or M65 biomarker and gender, age, or histopathological type.

A small sample size and short follow-up time were the major limitations of our study, but it is noteworthy that our results established serum M30 levels as an independent factor for predicting both OS and PFS in advanced-stage NSCLC. In the future, a larger sample size is needed to further determine the importance of serum M30 and M65 values as prognostic markers.

Acknowledgments This manuscript was not supported by any financial or other relationships.

Conflict of interest None.

References

1. Yaman E, Coskun U, Sancak B, Buyukberber S, Ozturk B, Benekli M. Serum M30 levels are associated with survival in advanced gastric carcinoma patients. Int Immunopharmacol. 2010;10:719–22.
2. Ueno T, Toi M, Linder S. Detection of epithelial cell death in the body by cytokeratin 18 measurement. Biomed Pharmacother. 2005;59:359–62.
3. Ueno T, Toi M, Bivén K, Bando H, Ogawa T, Linder S. Measurement of an apoptotic product in the sera of breast cancer patients. Eur J Cancer. 2003;39:769–74.
4. Kramer G, Erdal H, Mertens HJ, et al. Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. Cancer Res. 2004;64:1751–6.
5. Hickman JA, Beere HM, Wood AC, Waters CM, Parmar R. Mechanism of cytotoxicity caused by antitumor drugs. Toxicol Lett. 1992;64:553–61.
6. Ulukaya E, Karaagac E, Ari F, et al. Chemotherapy increases caspase-cleaved cytokeratin 18 in the serum of breast cancer patients. Radiol Oncol. 2011;45:116–22.
7. Demiray M, Ulukaya E, Arslan M, et al. Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. Cancer Invest. 2006;24:669–76.
8. Kramer G, Schwarz S, Hägg M, Havelka AM, Linder S. Docetaxel induces apoptosis in hormone refractory prostate carcinomas during multiple treatment cycles. Br J Cancer. 2006;94:1592–8.
9. de Haas EC, di Pietro A, Simpson KL, et al. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. Neoplasia. 2008;10:1041–8.
10. Olofsson MH, Ueno T, Pan Y, et al. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. Clin Cancer Res. 2007;13:3198–206.
11. Bilici A, Ustaalioglu BB, Ercan S, et al. Is there any impact of plasma M30 and M65 levels on progression-free survival of patients with advanced gastric cancer? Cancer Chemother Pharmacol. 2011;68:309–16.
12. Koelink PJ, Lamers CB, Hommes DW, Verspaget HW. Circulating cell death products predict clinical outcome of colorectal cancer patients. BMC Cancer. 2009;9:88.
13. Dive C, Smith RA, Garner E, et al. Considerations for the use of plasma cytokeratin 18 as a biomarker in pancreatic cancer. Br J Cancer. 2010;102:577–82.
14. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225–49.
15. Ulukaya E, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspasecleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. Lung Cancer. 2007;56:399–404.
16. Goldstraw P, Crowley J, Chansky K, et al. International association for the study of lung cancer international staging committee; participating institutions. The IASLC lung cancer staging project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol. 2007;2:706–14. Erratum in: J Thorac Oncol. 2007;2:985.
17. James K, Eisenhauer E, Christian M, et al. Measuring response in solid tumors: unidimensional versus bidimensional measurement. J Natl Cancer Inst. 1999;91:523–8.
18. Green AM, Steinmetz ND. Monitoring apoptosis in real time. Cancer J. 2002;8:82–92.
19. Hou JM, Greystoke A, Lancashire L, et al. Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am J Pathol. 2009;175:808–16.
20. Wu YX, Wang JH, Wang H, Yang XY. Study on expression of Ki-67, early apoptotic protein M30 in endometrial carcinoma and their correlation with prognosis [Article in Chinese]. Zhonghua Bing Li Xue Za Zhi. 2003;32:314–8.