The Role of MonoTotal in the Primary Diagnosis, Prognosis and Follow-up of Patients with Non-small Cell Lung Cancer (NSCLC)

MARKETA PRAZAKOVA[1,2], JINDRA VRZALOVA[1,2], JOSEP MARIA AUGE[3], JARMIL SAFRANEK[4], ONDREJ TOPOLCAN[1,2], RADKA FUCHSOVA[1,2], MARKETA SPISAKOVA[1], SARKA SVOBODOVA[1,6], LUBOS HOLUBEC JR.[5] and MARTIN PESTA[1]

[1]Laboratory of Immunoanalysis, Faculty of Medicine in Pilsen, Charles University in Prague, Czech Republic; Departments of [2]Nuclear Medicine, [4]Surgery and [5]Oncology, Faculty Hospital in Pilsen, Czech Republic; [3]Department of Biochemistry and Molecular Genetics, Hospital Clinic Barcelona, Spain; [6]Third Internal Medicine Department and First Medical Faculty, Charles University, Prague, Czech Republic

Abstract. *Background: A new cytokeratin tumor marker, MonoTotal was studied in lung cancer, the most common cause of cancer mortality worldwide. In non-small cell lung cancer (NSCLC) patients MonoTotal serum levels and their relationship to the tumor stage, histological subtype, early relapse and cancer-related death were evaluated. Patients and Methods: MonoTotal serum levels were studied, using immunoradiometric assay in a group of 93 patients with newly diagnosed NSCLC undergoing radical surgery, and were compared to those with benign lung diseases. Results: A diagnostic power of MonoTotal in distinguishing patients with NSCLC from benign lung diseases was demonstrated. Higher levels of MonoTotal were associated with advanced stages of squamous cell carcinoma and there was a positive correlation of marker with tumor size. Marker levels showed significant relation to disease-free survival and overall survival. Conclusion: MonoTotal seems to be a potentially very interesting serum marker that, in conjunction with other clinical data, might be used for monitoring of patients with NSCLC.*

Lung cancer is the most frequently diagnosed type of cancer in the world and the most common cause of cancer mortality worldwide, in the European Community lung cancer accounting for 29% of all cancer, and 21% of all male cancer (1). Epidemiological changes of lung cancer include the narrowing of the difference between men and women affected by the disease, increases in the incidence of adenocarcinoma histological subtypes, as well as more never-smokers affected by the disease (2-4).

Non–small cell lung cancer (NSCLC) accounts for about 80% of all lung cancer cases. Surgical resection offers the best chance for cure, but only 20-30% (stages I, II) of patients are candidates for surgical intervention, resulting in a 5-year survival rate in the early stages of aproximately 50-70% (5). Recent studies showed that survival of surgically resected patients with NSCLC might be improved by systemic platinum-based adjuvant chemotherapy especially in the early stages, but which patients might benefit from this treatment cannot be determined accurately (6-8). Therefore, there is growing need for diagnostic tools to estimate the prognosis of the patient, to monitor the treatment course, and to detect the response to therapy early, which would help to optimize the disease management on an individual basis, changing the treatment strategy early could then save time and costs, and avoid the patient being exposed to unnecessary side-effects.

In patients with lung cancer, several oncological biomarkers have shown considerable diagnostic and prognostic potential or have proven to be useful for the monitoring of systemic treatment and postoperative follow-up care (9-13). Among them, cytokeratins (CKs) are the most promising group as a complementary tool to other clinical methods in managing lung cancer patients. The clinical usefulness of CK serum markers is well established for monitoring patients with epithelial cell carcinomas during treatment and after therapy. At present, more than 20 different cytokeratins have been identified, of which CK 8, 18, and 19 are the most abundant cytokeratin proteins found in carcinomas. They are released from tumor cells and can be found in the blood, pleural effusions, cystic fluids, ascites

Correspondence to: Jindra Vrzalova, Immunoanalytic Laboratory, Department of Nuclear Medicine, Faculty Hospital in Pilsen, Dr. E. Benese 13, Plzen, CZ-30599, Czech Republic. E-mail: vrzalovaj@fnplzen.cz

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and urine. Upon release from proliferating or apoptotic cells, CKs provide useful markers for epithelial malignancies, distinctly reflecting tumor cell activity. By following up patients with repeated testing of a CK marker, the oncologist may obtain critical information regarding the tumor growth activity before that given by conventional methods (14). The three most applied CK markers used in the clinic are tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and Cytokeratin 19 Fragment (CYFRA 21-1). Recently, a new CK tumor marker MonoTotal, detecting fragments of CK 8, 18 and 19, has been introduced (15-17).

In the present study, we evaluated serum levels of MonoTotal in patients with newly diagnosed NSCLC to determine whether there is any relationships between serum MonoTotal levels and NSCLC stage, early relapse (local recurrence, distant metastasis or cancer-related death) and histological subtypes of NSCLC patients who underwent radical surgery.

Patients and Methods

Patients. MonoTotal was studied in 93 patients with newly diagnosed NSCLC who had undergone lung surgery (complete R0 resection) between 2004-2007 at the Department of Surgery, University Hospital in Pilsen. All patients were followed up until death or the last day of follow-up (December 31, 2009). The average length of follow-up was 28,2 month (range, 1-60 months). The following clinical parameters were studied: histological type of tumor, TNM classification, tumor stage, treatment strategy, tumor duplicity, first relapse, clinical status (remission, progression, stable disease) during follow-up and at the time of last examination, and cancer-related death. Clinical and laboratory examinations and imaging methods (X-ray, bronchoscopy, computed tomography, PET/CT scan, ultrasonography, and bone scan) were performed for all patients before start of therapy and during the follow-up period of at least half year. The response to therapy was classified according to the WHO classifications (18) defining complete remission as the disappearance of the tumor, partial remission as tumor reduction ≥50%, progression as tumor increase ≥25% or appearance of new tumor manifestations, and no change (stable disease) as tumor reduction <50% or increase <25%.

As a control group, 20 patients with benign lung disease (Table I) and no history of cancer disease were enrolled, whose median age (58 years, range 39-68 years) corresponded to the median age of the patients with NSCLC (62 years, range 43-77 years). At enrolment none of these individuals had renal failure, liver disease, or benign skin disease, well known causes of false-positive results of routine tumor markers.

The clinicopathologial characteristics of NSCLC patients and the control group are shown in Table II.

MonoTotal measurement. Levels of MonoTotal were measured in serum using MonoTotal immunoradiometric assay (IDL Biotech, Broma, Sweden) in accordance with the manufacturer's instructions. The monoclonal antibodies used in the MonoTotal assay are directed at epitopes on CK 8, 18 and 19; these epitope regions were described by Stigbrand *et al*. (19). Results are expressed as units per liter (U/l).

Table I. *Benign disease characteristics of the control group (N= 20).*

Benign disease	N
Postinflammatory fibrosis	5
Tuberculosis	4
Chondrohamartoma	4
Interstitial fibrosis	2
Lung fibroma	2
Lung granuloma	1
Bronchial cyst	1
Aspergiloma	1

In our study, samples of venous blood were collected prospectively prior to surgery and every 6 months during the follow-up period (*i.e*. at 6, 12, 18 and 24 months after surgery). After separation serum aliquots were stored at a temperature of –70°C until analysis. Patients were examined clinically after each blood sampling.

Statistical analysis. The MonoTotal baseline levels and levels during follow-up were evaluated for its power to discriminate between NSCLC and non malignancy, histological types and patients with progression and non progression by means of Wilcoxon rank sum test. To identify the best cut-off for differentiating between benign disease and NSCLC, and progression and remission, receiver operating characteristic (ROC) curves and corresponding areas under the curve (AUC) were calculated. In addition sensitivity calculated at 95% specificity, accompanied by 95% confidence intervals were calculated. To test the association of MonoTotal levels with disease-free survival (DFS) and overall survival (OS) of the patients, Cox proportional hazard regression model was used to evaluate the relation with the marker without dichotomization by cut-off. Additionally, Kaplan-Meier analysis of time-to-an-event and log-rank test for comparison of survival (OS or DFS) curve were used for the marker dichotomized by different cut-offs. The level of statistical significance of 5% (0.05) was used. All statistical analyses were carried out with SPSS for Windows software (version 15.0, 2006; SPSS Inc., Chicago, IL, USA) and SAS (version 9.1, SAS Institute, Cary, NC, USA).

Results

Pre-surgery levels of MonoTotal in benign group and NSCLC patients. Pre-surgery serum MonoTotal levels in benign group, NSCLC patients, and in relation to histological type, tumor stage and tumor size are presented in Table III and Figure 1. MonoTotal levels were significantly higher in the cancer group compared to the benign group (p-value=0.0028). In relation to histology, significantly higher levels of MonoTotal were found in squamous cell carcinoma compared to adenocarcinoma (p-value=0.0450). Higher levels of MonoTotal were associated with advanced stages of disease in squamous cell carcinoma (III *vs*. I, p-value=0.0140), but not in adenocarcinoma. Positive correlation was found

Table II. *The clinicopathological characteristics of NSCLC patients and the control group.*

		Study group NSCLC n (%)	Control group Benign disease n (%)
Total	93 (100)	20 (100)	
Age, years, median (range)	62 (43-77)	58 (39-68)	
Gender	Male	71 (76.4)	9 (45)
	Female	22 (23.6)	11 (55)
Smoker	Yes	86 (92.5)	13 (65)
	No	7 (7.5)	7 (35)
Histology	Adenocarcinoma	34 (36.6)	
	Squamous	59 (63.4)	
Stage	Ia	19 (20.4)	
	Ib	30 (32.3)	
	IIa	4 (4.3)	
	IIb	15 (16.1)	
	IIIa	25 (26.9)	
Follow-up	Disease-free survival		
Mean (range) months	34.1 (28.5-39.7)		
	Overall survival		
Mean (range) months	41.4 (36.2-46.5)		
Initial therapy	No therapy	23 (24.7)	
	Adjuvant	56 (60.2)	
	Palliative	14 (15.1)	
Therapy response	Remission	45 (48.4)	
	Stable disease	5 (5.4)	
	Progression	43 (46.2)	
Survival at study end (Dec. 31, 2009)	Survivors	48 (51.6)	
	Cancer death	45 (48.4)	

between pre-surgery MonoTotal levels and tumor size (T) with significantly higher levels in larger tumors (T4) compared to smaller ones (T1) in group of NSCLC patients (p-value=0.0077) and in squamous cell carcinoma subgroup (p-value=0.0240).

Results of the ROC evaluation are shown in Table IV. MonoTotal showed the best accuracy to distinguish patients with squamous cell carcinoma and benign lung disease, with cut off of 200.7 U/l at 95% specificity.

Postsurgery follow- up monitoring of NSCLC patients. The biomarker results during follow-up monitoring of NSCLC patients were divided into the remission and progression subgroups according to the clinical status of the patients at the time of blood sampling. Median levels of MonoTotal were significantly higher in NSCLC patients with progression (median=141.8 U/l, range 39.4-2632 U/l) compared to those with remission (median=63.7 U/l, range below detection – 383.3 U/l). See Figure 2 for box-plot of remission and progression values.

The AUC in ROC analysis of follow-up results was 0.8. The sensitivity at 95% specificity was 52% with cut-off of 161 U/l, the positive predictive value was 64.7% and the negative predictive value was 90.2%.

Table III. *Descriptive statistics – Serum MonoTotal levels in patient groups and subgroups.*

	Median	Mean	SD	Minimum	Maximum
Benign	93.0	98.8	55.9	22.3	218.3
NSCLC	155.2	261.2	361.1	10.4	2167.8
Stage I	131.6	208.2	319.1	10.4	2167.8
Stage II	160.7	300.8	373.5	19.8	1435.0
Stage III	184.8	339.6	425.7	59.8	1861.8
Adenocarcinoma	125.4	182.7	251.6	10.4	1435.0
Squamous cell carcinoma	165.7	304.0	404.4	23.6	2167.8
T1	124.4	214.5	442.2	10.4	2167.8
T2	163.9	242.3	267.4	36.2	1435.0
T3	165.1	208.6	121.5	84.9	404.3
T4	234.7	537.7	590.3	72.4	1861.8

SD=Standard deviation.

The prognostic value of MT: relation with DFS and OS of patients with NSCLC. MonoTotal showed a significant relation with DFS and OS in NSCLC patients (Figures 3 and 4). For DFS, p-value by means of Cox model was <0.0001 and after dichotomization by cut-off 200 U/l, p-value of log-rank test was 0.0113. For OS in the Cox model by means, p-

Table IV. *ROC characteristics of MonoTotal levels in NSCLC compared to benign group and separately in adenocarcinoma and squamous cell carcinoma subgroups compared to benign group.*

	Sensitivity at 95% specificity	AUC	Cut-off (U/l)	PPV (%)	NPV (%)
NSCLC × benign	32.9	0.73	200.7	96.6	21.9
Adenocarcinoma × benign	26.7	0.65	206.0	88.9	42.1
Squamous cell carcinoma × benign	36.4	0.78	200.7	95.2	30.8

AUC=Area under ROC curve; PPV=positive predictive value; NPV=negative predictive value.

value was <0.0001 and after dichotomization by cut-off 200 U/l, *p*-value of log-rank test was 0.0018. Univariate analysis according to the histological subtype of NSCLC showed MonoTotal as prognostic factor in squamous cell carcinoma for both DFS and OS (*p*-value<0.0001), but in adenocarcinoma only for DFS (*p*-value=0.0317).

Discussion

Our work was focused on MonoTotal, a new CK-based tumor marker utilizing a combination of three monoclonal antibodies directed against soluble fragments of CK 8, 18 and 19. There is a lack of published data on this novel marker. One of the existing studies demonstrate its utility in esophageal carcinoma (15), with the authors showing correlation to increased tumor burden and concluding that this marker might, in conjunction with other clinical parameters, help the clinician in estimating the prognosis of the individual patient for this diagnosis.

There is only one published study showing its usefulness in NSCLC (17). The authors performed a study only for patients in stage III disease in contrast to our study incorporating stages I III. Our results, confirm the study of Eriksson *et al*. (17). We found in stage III pre-surgery median levels of 184.8 U/l compare to 207 U/l pretreatment levels published by Eriksson *et al*. Furthermore, we observed significantly higher levels of MonoTotal in squamous cell carcinoma than that of adenocarcinoma in concordance with the cited study. Both studies also confirm the correlation of MonoTotal with tumor volume. In our study, the pre surgery levels were also correlated to tumor burden. These results support the notion of MonoTotal as being a marker of biological activity of tumor as was proposed for other markers from the CK group (14).

In our study, analysis of ROC curves (AUC=0.73) demonstrated a diagnostic accuracy of MonoTotal in distinguishing patients with NSCLC from those with benign lung disease. We found a sensitivity for diagnosis of 32.9% at 95% specificity with related cut-off value of 200.7 U/l. This lack of sensitivity in our study might be due to the high proportion of early lung cancer stages (I IIb) of the enrolled patients. We could compare our data to that for CYFRA 21-1,

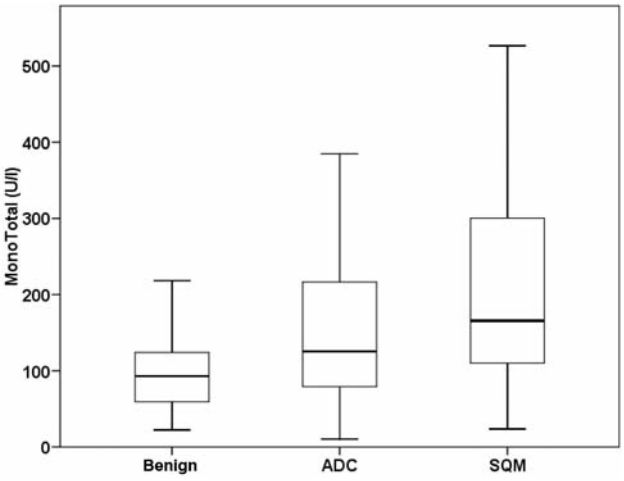

Figure 1. *Pre-surgery MonoTotal levels in benign vs. NSCLC histology subgroups. ADC: Adenocarcinoma NSCLC; SQM:squamous cell carcinoma NSCLC.*

which is the most studied CK marker in NSCLC. In Nisman *et al*. study incorporating patients with I-IV stage, CYFRA 21-1 showed an AUC of 0.84. Even if the advanced stages were enrolled in high numbers cited in the study, the sensitivity of only 49.5% at 95% specificity for benign lung diseases was published (20). Several studies imply that the lack of sensitivity is common for all CKs in NSCLC, which is why the CKs are not suitable in screening for lung cancer (14).

Several recent studies have suggested that CK 8, 18 and 19 have a role in tumor progression. The increased expression of these CKs was found during progression of some human tumors, including lung cancer (21). The usefulness of MonoTotal for detection of disease progression during follow-up is shown in our study by a sensitivity of 52% at 95% specificity. We suggest that MonoTotal might be considered as an early indicator of relapse during follow-up in NSCLC patients. Changes of MonoTotal often preceeded detection of relapse by other conventional methods. Increasing of MonoTotal levels during follow-up should indicate more rigorous examination of patients, including imaging methods. This could help in early diagnosis and treatment of tumor relapse. Our observations

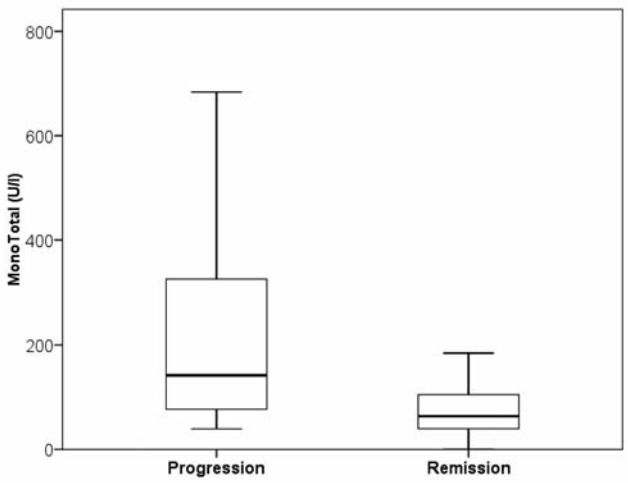

Figure 2. *MonoTotal levels related to remission and progression during follow-up.*

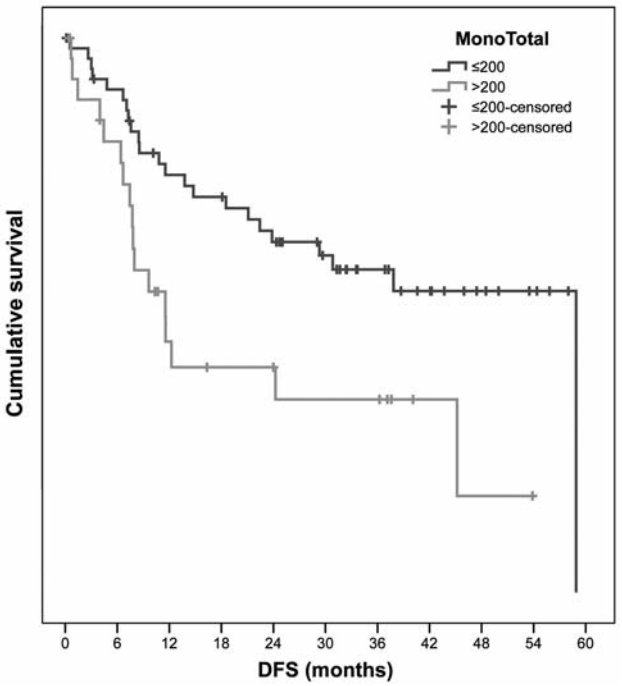

Figure 3. *Disease-free survival (DFS) rate in NSCLC patients according to the preoperative serum MonoTotal level. Dichotomization by cut-off of 200 U/l.*

complement the conclusions published by Eriksson *et al*. (17) showing a decrease of MonoTotal levels during treatment and their strong increase in disease progression after treatment failure until time of death of patients.

Preoperative MonoTotal serum levels seem to be of prognostic interest in NSCLC patients. In our study, both DFS and OS demonstrated that NSCLC, squamous and adenocarcinoma patients with preoperative MonoTototal levels >200 U/l had a significantly unfavorable prognosis. We found that serum MonoTotal level at the time of diagnosis was a reliable predictor of DFS and OS, a high value being associated with worse prognosis. Our findings suggest that in completely resected NSCLC, preoperative serum MonoTotal levels might provide a useful tool for stratifying subgroups of patients with different chances of disease recurrence after surgery. In the study of Eriksson *et al*., levels of MonoTotal were found to be associated with survival in stage IIIb patients (17).

In our opinion, MonoTotal may be used as an aid in the diagnosis of NSCLC patients, for early detection of relapse and as a survival factor. MonoTotal seems to be promising marker for prognosis.

A CK marker assay should be performed both before treatment (to exploit the capability for giving insight into the severity of the illness and its possible outcome), and, serially, during and after treatment (to help decide on the status of the disease and its response to the treatment). While there are mostly comparable results among CKs and because they all reflect the same biological process they should be combined not together but with the markers from another group. MonoTotal seems to be a potentially very interesting serum marker that, in conjunction with other clinical data, might be used for monitoring of patients with NSCLC.

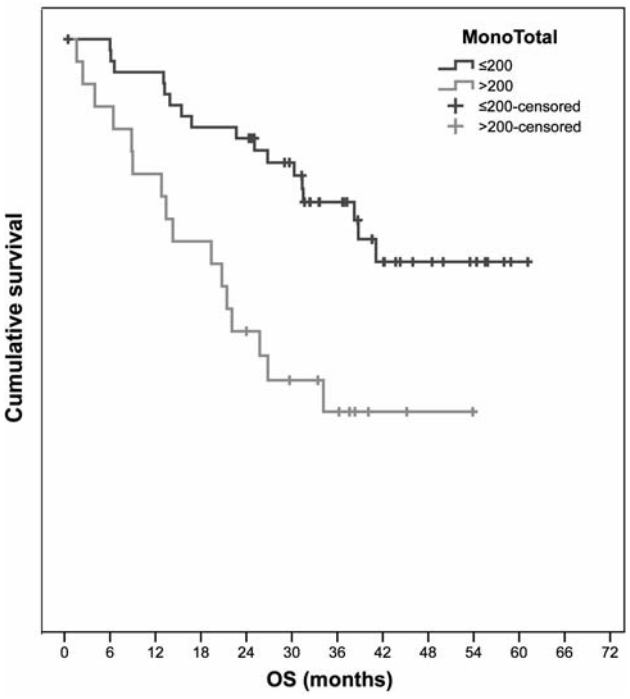

Figure 4. *Overall survival (OS) rate in NSCLC patients according to the preoperative serum MonoTotal level. Dichotomization by cut-off of 200 U/l.*

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