Prognostic Significance of Serum Tumor Markers in Patients with Advanced-stage NSCLC Treated with Pemetrexed-based Chemotherapy

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Abstract. *Background: Tumor biomarkers represent effective tools for diagnostics and follow-up monitoring of patients with non-small cell lung cancer (NSCLC). We focused on evaluating the predictive and prognostic role of the seven following tumor biomarkers: carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA 21-1), fragments of cytokeratin 8, 18 and 19 (MonoTotal), neuron-specific enolase (NSE), chromogranin A, thymidine kinase (TK) and squamous cell carcinoma antigen (SCCA) in patients with advanced-stage NSCLC treated with pemetrexed-based chemotherapy. Patients and Methods: In total, 114 patients with advanced-stage (IIIB or IV) non-squamous NSCLC treated with pemetrexed-based chemotherapy (monotherapy or combination with a platinum derivative) were included. Comparison of progression-free (PFS) and overall survival (OS) according to the level of assessed tumor markers was performed using the log-rank test. Results: We recorded significantly shorter OS for patients with high pretreatment levels of CYFRA 21-1 (10.3 vs. 23.4 months; p<0.001), NSE (1.6 vs. 13.5 months; p=0.003) and TK (11.3 vs. 23.4 months; p=0.003). Conclusion: CYFRA 21-1, NSE and TK are feasible biomarkers for estimation of a patient's overall prognosis, however, none of the measured serum tumor markers were able to predict the efficacy of pemetrexed-based chemotherapy.*

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Lung cancer is the principal cause of cancer-related death worldwide and its incidence is still increasing (1). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, constituting more than 80% of all lung carcinomas (2). Pemetrexed represents one of the new effective agents approved for the treatment of advanced-stage NSCLC in recent years.

The aim of the present study was to evaluate the prognostic and predictive role of pretreatment serum levels of seven tumor markers, namely: carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA 21-1), fragments of cytokeratin 8, 18 and 19 (MonoTotal), neuron-specific enolase (NSE), chromogranin A, thymidine kinase (TK) and squamous cell carcinoma antigen (SCCA) in patients with advanced-stage NSCLC treated with pemetrexed-based chemotherapy.

Patients and Methods

Patients' characteristics. The study included 114 patients. The median age was 64 years (range=28-83 years). A total of 67 (58.8%) patients were males, 91 (79.8%) patients had a positive smoking history, 103 (90.4%) patients had adenocarcinoma, 87 (76.3%) patients had stage IV disease, 96 (84.2%) patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 and 72 (63.2%) patients were treated with a combination of pemetrexed and platinum derivative. The baseline patient characteristics are summarized in Table I.

Study design and treatment. We retrospectively analyzed clinical and laboratory data of patients with cytologically- or histologically-confirmed advanced-stage (stage IIIB or IV) non-squamous NSCLC treated with pemetrexed-based chemotherapy between 2008 and 2014 at the Department of Pneumology in Pilsen.

Pemetrexed was administered intravenously at the standard approved dose of 500 mg/m^2 in monotherapy (mostly in first-line setting), or in combination (mostly in pretreated patients) with

platinum derivative cisplatin at a dose of 75 mg/m^2 or carboplatin at area under the concentration–time curve (AUC) 6 on day 1 every 3 weeks. Treatment with platinum derivative was scheduled for up to four cycles, followed by maintenance pemetrexed monotherapy in patients with non-progressive disease and treatment with pemetrexed monotherapy was maintained until disease progression occurred, unless there was development of intolerable toxic effects.

Clinical monitoring. Treatment was prospectively monitored and the clinical course of patients was continuously assessed at specific time points. Clinical follow-up including physical examination, plain chest X-ray and routine laboratory tests was performed every 3-4 weeks; computed tomography (CT) or positron-emission tomography-CT (PET-CT) was performed after two or three cycles.

Data source. The clinical registry TULUNG (http://tulung.registry.cz/), in which Faculty of Pilsen participates, is a non-interventional post-registration database of epidemiological and clinical data of patients with advanced-stage NSCLC treated with targeted therapy in the Czech Republic. The registry contains anonymised individual patient data including demographic parameters, initial staging and disease characteristics, baseline patient information at the start of targeted therapy, as well as data on survival and adverse events, and is updated at least twice a year. Data on tumor markers were not recorded in this registry therefore we extracted these values from the hospital information system and merged it with the registry data.

Tumor marker measurement. Serum samples for measurement of serum tumor markers were collected within one month before erlotinib treatment. Serum levels of CEA were measured using a chemiluminiscent method on a DXI 800i analyzer (Beckman Coulter, CA, USA). Serum levels of CYFRA 21-1 and NSE were measured using an immunoradiometric titration method (IRMA) on a Stratec 300 analyzer (Immunotech, Prague, Czech Rep.). Serum levels of TK were measured using radioenzymatic assay on a Stratec 300 analyzer (Immunotech). Serum levels of chromogranin A were measured using IRMA on a Stratec 300 analyzer (Cisbio, Codolet, France). Serum levels of MonoTotal were measured using IRMA on a Stratec SR 300 (IDL Biotech, Bromma, Sweden). Serum levels of SCCA were measured using chemiluminiscent method on an Architect i 1000 analyzer (Abbott Laboratories, Wiesbaden, Germany).

Measurements were made at the Central Immunoanalytic Laboratory at the Department of Nuclear Medicine, using the following cut-off values: CEA: 3 μ g/l; CYFRA 21-1: 2.5 μ g/l; NSE: 12.5 μ g/l; TK: 8 U/l; chromogranin A: 100 μ g/l; MonoTotal: 100 U/l; and SCCA: 2.5 μg/l.

Statistical analysis. Standard frequency tables and descriptive statistics were used to characterize the sample data set. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method and all point estimates were accompanied by 95% confidence intervals. PFS was determined from the date of pemetrexed initiation until the date of first documented progression or death. OS was determined from the date of pemetrexed initiation until the date of death. Statistical significance of the differences in Kaplan–Meier estimates according to tumor marker levels was assessed using the log-rank test. Multivariable Cox proportional hazards model was used to adjust results in terms of tumor markers on stage at treatment initiation and combination therapy as the most clinical relevant potential confounders. As a level of statistical significance, α=0.05 was used.

Results

Tumor marker levels before treatment. Before the begining of pemetrexed-based chemotherapy treatment, high value for CEA (≥3 μg/l) was measured in 85 (74.6%) patients, CYFRA 21-1 (≥2.5 μg/l) in 57 (50.0%), MonoTotal (≥100 U/l) in 44 (51.2%) patients, NSE (≥12.5 μg/l) in eight (8.5%), chromogranin A (≥100 μ g/l) in 18 (26.9%) patients, TK (≥8 U/l) in 54 (47.4%), and for SCCA (≥2.5 μg/l) in 19 (21.8%) patients.

Relation between levels of tumor markers and treatment efficacy. For patients with high CEA, the median PFS and OS were 2.4 and 12.8 compared to 3.1 and 11.5 months for patients with low CEA (p=0.137 and p=0.866). For patients with high CYFRA 21-1, the median PFS and OS were 2.4 and 10.3 compared to 2.7 and 23.4 months for patients with low CYFRA 21-1 (p=0.899 and p=0.001) (Figure 1A). For patients with high MonoTotal, the median PFS and OS were 3.1 and 12.4 compared to 2.9 and 22.3 months for patients with low MonoTotal (p=0.543 and p=0.451). For patients with high NSE, the median PFS and OS were 1.3 and 1.6 compared to 2.8 and 13.5 months for patients with low NSE (p=0.392 and p=0.003) (Figure 1B). For patients with high chromogranin A, the median PFS and OS were 1.4 and 7.9 compared to 2.4 and 13.5 months for patients with low chromogranin A (p=0.196 and p=0.224). For patients with high TK the median PFS and OS were 2.2 and 11.3 compared to 2.8 and 23.4 months for patients with low TK (p=0.163 and p=0.003) (Figure 1C). For patients with high SCCA, the median PFS and OS were 2.7 and 8.7 compared to 2.9 and 16.6 months for patients with low SCCA (p=0.557 and p=0.510). All survival data are summarized in Table II. The multivariate Cox proportional hazards model revealed high pretreatment serum levels of CYFRA 21-1 [hazard ratio (HR)=2.26, p=0.001], NSE (HR=4.20, p=0.005) and TK (HR=2.09, p=0.003) as significant independent factors predictive of poor OS (Table III).

Discussion

Pemetrexed is an intravenously administered antifolate cytostatic targeting several folate-dependent enzymatic pathways. Phase III clinical trials demonstrated efficacy of pemetrexed in combination with platinum derivative (*i.e.* cisplatin or carboplatin) in the first-line treatment of patients with advanced-stage non-squamous NSCLC (3, 4) and also as a single-agent in previously treated patients (5). Recently, the phase III clinical trial PARAMOUNT proved efficacy of pemetrexed in the maintenance setting (6). CEA, CYFRA 21-1, MonoTotal, NSE, chromogranin A, TK and SCCA are

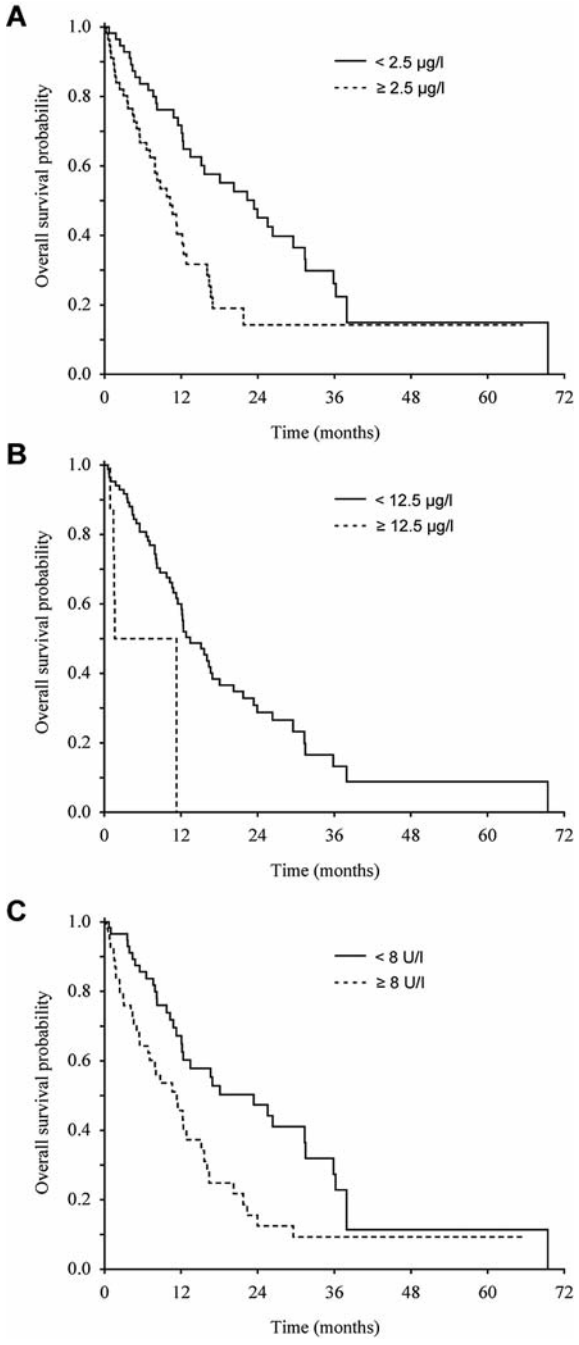

Figure 1. *Kaplan–Meier plots showing the comparison of overall survival according to pretreatment serum cytokeratin-19 fragment (A), neuron-specific enolase (B) and thymidine kinase (C) levels.*

serum tumor markers feasible for use in diagnostics and follow-up monitoring of patients with NSCLC. In the present study, we focused on their potential prognostic and predictive role in patients with advanced-stage NSCLC treated with pemetrexed-based chemotherapy.

Table I. *Baseline patient characteristics.*

	Total (n=114)
Gender, n (%)	
Male	67 (58.8)
Female	47 (41.2)
Age, years	
Median (min-max)	64 (28-83)
Smoking history, n (%)	
Current smoker	60 (52.6)
Former smoker	31 (27.2)
Never smoker	23 (20.2)
Histology, n (%)	
Adenocarcinoma	103 (90.4)
Other non-squamous	11 (9.6)
Stage, n (%)	
IIIB	27 (23.7)
IV	87 (76.3)
Performance status, n (%)	
0	18 (15.8)
1	96 (84.2)
Line of therapy, n (%)	
1st	72 (63.2)
2nd	31 (27.2)
3rd and 4th	11 (9.6)
Combination with platinum derivative, n (%)	
Yes	72 (63.2)
No	42 (36.8)

CEA plays a role in cell-to-cell adhesion and has and important role in blocking cell differentiation (7) and it cooperates with v-myc avian myelocytomatosis viral oncogene homolog (MYC) and B-cell lymphoma 2 protein (BCL2) in cellular tranformation (8). In our study, we did not observe any significant difference in PFS nor in OS between patients with high compared to those with low CEA levels, however, a high CEA level has been described as a significant marker of poor prognosis in patients with NSCLC regardless of treatment (9, 10). Our results are in agreement with those recently published by Liu *et al*., who focused on the role of CEA in predicting response to chemotherapy in 689 patients with NSCLC treated with different chemotherapy regimens (11). CYFRA 21-1, a fragment of cytokeratin subunit 19, has been extensively evaluated in NSCLC. In our study, we recorded significantly shorter OS for patients with a high CYFRA 21-1 level compared to those with a low CYFRA 21-1 level, while there was no significant difference in PFS. The multivariate Cox proportional hazards model revealed a high pretreatment level of CYFRA 21-1 to be an independent factor predictive of poor OS (HR=2.26, p=0.001).

It is interesting to compare data of the present study to our previously published work focused on the predictive role of the pretreatment serum levels of CEA and CYFRA 21-1 in

Table II. *Progression-free (PFS) and overall (OS) survival from pemetrexed-based chemotherapy treatment initiation according to tumor marker level.*

Marker	n (%)	Median PFS (95% CI), months	*p*-Value*	Median OS (95% CI), months	*p*-Value*
CEA					
<3.0 μg/l	29 (25.4)	3.1 (0.7-5.4)	0.137	11.5 (1.4-21.5)	0.866
≥3.0 μg/l	85 (74.6)	2.4 (1.4-3.4)		12.8 (8.5-17.2)	
CYFRA 21-1					
<2.5 μg/l	57 (50.0)	2.7 (0.7-4.7)	0.899	23.4 (14.7-32.1)	0.001
≥2.5 μg/l	57 (50.0)	2.4 (1.8-2.9)		10.3 (6.8-13.7)	
MonoTotal					
<100 U/l	42 (48.8)	2.9 (1.1-4.8)	0.543	22.3 (5.0-39.7)	0.451
≥100 U/l	44 (51.2)	3.1 (1.5-4.6)		12.4 (5.4-19.4)	
NSE					
<12.5 μg/l	86 (91.5)	2.8 (2.1-3.4)	0.392	13.5 (9.6-17.3)	0.003
≥12.5 μg/l	8 (8.5)	1.3 (0.8-1.9)		1.6 (0.1-7.1)	
Chromogranin A					
<100 μg/l	49 (73.1)	2.4 (1.4-3.4)	0.196	13.5 (7.1-19.8)	0.224
≥100 μg/l	18 (26.9)	1.4 (1.3-1.5)		7.9 (0.1-18.0)	
TK					
<8 U/l	60 (52.6)	2.8 (2.1-3.5)	0.163	23.4 (11.5-35.2)	0.003
≥8 U/l	54 (47.4)	2.2 (1.2-3.3)		11.3 (6.7-15.9)	
SCCA					
<2.5 μg/l	68 (78.2)	2.9 (2.2-3.7)	0.557	16.6 (5.6-27.7)	0.510
≥2.5 μg/l	19 (21.8)	2.7 (0.1-7.4)		8.7 (5.1-12.4)	

CEA: Carcinoembryonic antigen; CYFRA 21-1: cytokeratin-19 fragmen; MonoTotal: fragments of cytokeratin 8, 18 and 1; NSE: neuron-specific enolase; TK: thymidine kinas; SCCA: squamous cell carcinoma antigen. *Log-rank test.

patients with advanced-stage NSCLC treated with the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) erlotinib, where we found that high pretreatment serum levels of CEA and CYFRA 21-1 independently predicted short OS and PFS (12). On the other hand, in the present study focused on patients treated with pemetrexed-based chemotherapy, pretreatment serum levels of CEA and CYFRA 21-1 did not predict PFS. Thus CEA and CYFRA 21-1 levels could be useful biomarkers for optimal treatment choice, especially between EGFR-TKIs and pemetrexed in patients harboring wild-type *EGFR* gene, or those with unknown *EGFR* gene mutation status.

MonoTotal is a novel cytokeratin tumor marker, detecting fragments of cytokeratin 8, 18 and 19 (13). There is a lack of published data on this tumor marker. In our study, we observed no significant difference in PFS nor in OS between patients with high MonoTotal levels and those with low MonoTotal levels. Prazakova *et al*. reported MonoTotal to be a negative prognostic factor in patients undergoing lung surgery for NSCLC (14), however, there exist no data on the prognostic or predictive role of MonoTotal in patients with advanced NSCLC treated with chemotherapy.

NSE is a glycolytic enzyme largely expressed in neuroendocrine tumors. It is not commonly expressed in NSCLC, except for a subset of 10-20% cases with neuroendocrine differentiation (15). In our study, OS was significantly shorter for patients with a high NSE level compared to those with low NSE levels, while there was no significant difference in PFS, although we noticed a trend for shorter PFS in those with a high NSE level. This finding was confirmed by the multivariate Cox proportional hazards model.

Chromogranin A is a precursor of several functional peptides. It is another marker of neuroendocrine differentiation in NSCLC. In our study, there was no significant difference in PFS or OS between patients with a high chromogranin A level and those with low levels, although we noticed a trend for shorter survival (PFS, OS) in patients with a high chromogranin A level. Thus our results are in discordance with several previously published studies suggesting that the expression of neuroendocrine markers could predict better response to chemotherapy in NSCLC patients (16-18), however, these studies were not specifically focused on pemetrexed-based chemotherapy.

TK is an enzyme indicating proliferative characteristics of the cell. There are two isoforms, TK I and TK II, different in chemical structure and biological function. TK I is the most important, commonly used for detection and estimation of prognosis in cancer. TK I appears during cell division in the G_1 and S phase, while it is absent from resting cells (19).

Table III. *Results of seven multivariable Cox models for progression-free (PFS) and overall (OS) survival.*

	HR (95% CI)						
Factor	CEA	CYFRA 21-1	NSE	TK	Chromogranin A	MonoTotal	SCCA
PFS							
Serum level ≥Cut-off *vs.* <	1.46 (0.91-2.33)	1.10 (0.74-1.63)	1.25 (0.53-2.97)	1.18 (0.78-1.78)	1.27 (0.65-2.47)	0.92 (0.58-1.45)	0.89 (0.50-1.58)
Stage IV *vs.* IIIB	1.29 (0.79-2.10)	1.34 (0.82-2.18)	1.19 (0.69-2.03)	1.27 (0.77-2.11)	1.26 (0.66-2.40)	1.44 (0.81-2.56)	1.48 (0.84-2.61)
Combination with CT Yes *vs.* no	0.72 (0.47-1.09)	0.75 (0.49-1.13)	0.81 (0.51-1.29)	0.78 (0.51-1.19)	0.89 (0.48-1.65)	0.70 (0.42-1.19)	0.73 (0.43-1.23)
OS							
Serum level ≥Cut-off *vs.* <	1.05 (0.60-1.82)	2.26 (1.38-3.71)***	4.20 (1.53-11.50)**	2.09 (1.28-3.40)**	1.55 (0.77-3.09)	1.28 (0.70-2.34)	1.30 (0.61-2.76)
Stage IV *vs.* IIIB	0.98 (0.56-1.75)	1.11 (0.62-1.97)	0.78 (0.42-1.45)	0.98 (0.55-1.74)	0.63 (0.31-1.29)	1.09 (0.54-2.21)	1.05 (0.53-2.09)
Combination with CT Yes *vs.* no	0.95 (0.58-1.55)	0.94 (0.57-1.55)	1.06 (0.61-1.84)	1.12 (0.67-1.86)	0.92 (0.48-1.75)	0.98 (0.49-1.94)	0.96 (0.49-1.89)

CEA: Carcinoembryonic antigen; CYFRA 21-1: cytokeratin-19 fragmen; MonoTotal: fragments of cytokeratin 8, 18 and 1; NSE: neuron-specific enolase; TK: thymidine kinas; SCCA: squamous cell carcinoma antigen. Log-rank test: *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$; all other data, $p > 0.05$.

In our study, OS was significantly shorter for patients with a high TK level compared to those with low TK levels, while there was no significant difference in PFS. The multivariate Cox proportional hazards model confirmed this. Our results are similar to several previously published studies showing a high TK level to be a negative prognostic factor for NSCLC (20, 21).

SCCA is a purified sub-fraction of a tumor-associated antigen originally isolated from squamous cell carcinoma of the uterine cervix. Elevated serum SCCA levels were also found in patients with squamous cell carcinoma of the bronchus, nasopharynx and tumors of other histological origin (22). High SCCA levels are not common in patients with non-squamous cell lung cancer, except for a subset of approximately 20% cases. In our study, there was no significant difference in PFS or OS between patients with a high SCCA level and those with low SCCA levels. Body et al. previously reported a high SCCA level to be a negative prognostic factor for patients with squamous cell lung cancer (23), but there are currently no data on patients with non-squamous NSCLC, especially those with advanced-stage disease treated with chemotherapy.

The principal limitations of our study are the relatively small number of patients included and its retrospective design.

In conclusion, this is the first study focusing on the prognostic and predictive role of the serum tumor markers CEA, CYFRA 21-1, NSE, TK, chromogranin A, MonoTotal and SCCA in patients with advanced-stage NSCLC treated with pemetrexed-based chemotherapy. The study shows that high serum levels of CYFRA 21-1, NSE and TK are feasible biomarkers for estimation of a patient's overall prognosis, however, none of these markers was specifically able to predict the efficacy of pemetrexed-based chemotherapy in terms of PFS.

Conflicts of Interest

JF has received honoraria from Astra Zeneca, Roche and Novartis for consultations and lectures unrelated to this project. OF, MP, MS, OS, ZB, RK and OT declare that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence this work.

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