Prognostic Role for CYFRA 21-1 in Patients With Advanced-stage NSCLC Treated With Bevacizumab Plus Chemotherapy

MARTIN SVATON[1], JIRI BLAZEK[1], GABRIELA KRAKOROVA[1], MILOS PESEK[1],
MARCELA BURESOVA[1], ZUZANA TEUFELOVA[1], JOSEF VODICKA[2],
KAROLINA HURDALKOVA[3], MAGDA BARINOVA[3] and ONDREJ TOPOLCAN[4]

[1]Department of Pneumology and Phthisiology, Charles University,
Faculty of Medicine in Pilsen, Pilsen, Czech Republic;
[2]Department of Surgery, Charles University, Faculty of Medicine in Pilsen, Pilsen, Czech Republic;
[3]Institute of Biostatistics and Analyses, Ltd., Brno, Czech Republic;
[4]Department of Nuclear Medicine, Charles University, Faculty of Medicine in Pilsen, Pilsen, Czech Republic

Abstract. *Aim: To investigate potential associations between selected oncomarkers [carcinoembryonic antigen (CEA), C-terminus of cytokeratin 19 (CYFRA 21-1, CYFRA), and squamous cell carcinoma antigen (SCC)] and outcomes in patients with NSCLC treated with bevacizumab plus chemotherapy. Patients and Methods: We retrospectively analysed 105 patients with NSCLC from the Czech TULUNG registry treated at University Hospital in Pilsen with bevacizumab plus chemotherapy. Response to therapy was tested by Fisher's exact test. Survival statistics were evaluated using the Kaplan–Meier method and Cox analysis. Results: Only normal values of CYFRA (not CEA or SCC) were associated with significantly better overall and progression-free survival in univariate analysis. We also observed a trend for a better disease control rate in patients with normal levels of CYFRA. In a multivariate Cox model, only CYFRA was associated with significantly better overall but not progression-free survival. Conclusion: In our retrospective study, we point out the possibility of using CYFRA as a prognostic marker in patients with NSCLC treated with chemotherapy plus bevacizumab.*

Bevacizumab is an intravenously administered monoclonal antibody targeting vascular endothelial growth factor that is widely used in treating patients with advanced non-small cell lung cancer (NSCLC) (1). The Eastern Cooperative Oncology Group (ECOG) 4599 phase III trial showed a significant survival benefit with the use of bevacizumab in combination with carboplatin and paclitaxel compared to these alone in patients with previously untreated advanced, metastatic, or recurrent NSCLC (2). Such results were achieved using bevacizumab as maintenance therapy until progressive disease. It has been demonstrated that the superiority of bevacizumab is limited to patients with non-squamous histology due to a higher proportion of potentially risky haemoptysis in squamous lung cancer (3). Aside from the non-squamous histology, there is still no molecular biomarker available for predicting treatment efficacy of bevacizumab-based therapy.

A number of past studies have endeavoured to find an effective predictive marker for such treatment (4-7). In particular, investigations examined the potential for using expression of vascular endothelial growth factor, the effect of arterial hypertension, or measures in perfusion computed tomography (CT) to determine the effect of angiogenesis in a given tumour (4-6). However, none of these markers was reliable enough or subsequently verified sufficiently by prospective work to be put into routine clinical practice (3). Oncomarkers have shown potential as predictors of treatment outcomes in other NSCLC studies (8, 9). Given this background, the aim of the present study was to investigate potential associations between selected oncomarkers and outcomes in patients with NSCLC treated with bevacizumab plus chemotherapy.

Patients and Methods

Study design and treatment. Clinical data of patients with cytologically or histologically confirmed advanced NSCLC treated with bevacizumab and chemotherapy (mainly CP) between 2010 and 2020 at the Department of Pneumology and Phthisiology, University Hospital Pilsen, Czech Republic, were retrospectively analysed.

Correspondence to: Martin Svaton, Department of Pneumology and Phthisiology, Charles University, Faculty of Medicine in Pilsen, Husova 3, 301 00, Pilsen, Czech Republic. Tel.: +420 377402973, e-mail: svatonm@fnplzen.cz

Bevacizumab and chemotherapy treatment was undertaken in the first line (rarely second line). Bevacizumab was administered intravenously at the approved dose of 7.5 mg/kg every 3 weeks together with platinum doublet chemotherapy. The treatment with bevacizumab was administered until disease progression or unacceptable toxicity. Chemotherapy was given up to four cycles. Clinical follow-up including physical examination, chest X-ray, and routine laboratory tests was conducted at least every 4 weeks. CT or positron-emission tomography/CT was performed at regular intervals according to the local standards or when progression was suspected based on clinical or chest X-ray examination. Oncomarkers investigated in the present study included carcinoembryonic antigen (CEA), C-terminus of cytokeratin 19 (CYFRA 21-1, CYFRA), and squamous cell carcinoma antigen (SCC) measured at the initiation of bevacizumab plus chemotherapy. The TULUNG national register, a non-interventional post-registration database of epidemiological and clinical data of patients with advanced-stage NSCLC treated with targeted or biological therapies in the Czech Republic, served as the data source. We used data recorded from our centre (University Hospital). The patients had given their informed consent to be included in this database and for use of these data for scientific purposes.

Statistical methods. Standard frequency tables and descriptive statistics were used to characterize the sample data set. The overall response rate (ORR) was defined as the best response according to the Response Evaluation Criteria in Solid Tumours (RECIST 1.1) (10). Continuous parameters are described using the mean with 95% confidence interval, and the median with minimum and maximum, together with the total number of observations. Categorical parameters were summarized using absolute and relative frequencies. Relative frequencies were calculated based on the number of patients in the relevant subgroups. The ORR was tested by Fisher's exact test. Overall survival (OS) was defined as the time from treatment initiation to the date of death due to any cause. Progression-free survival (PFS) was defined as the time from treatment initiation to the date of first documented progression or death due to any cause. OS and PFS were estimated using the Kaplan–Meier method, and all point estimates include 95% confidence intervals. Differences between OS and PFS were tested by log-rank test. Finally, a multivariate Cox proportional hazards model was used to evaluate the effect of all potential prognostic factors on the survival measures. Statistical significance was determined at the level of $\alpha = 0.05$.

Cut-offs for oncomarkers were set at the normal range *versus* elevated value (*i.e.* 0-3 µg/l or higher for CEA, 0-2.5 µg/l or higher for CYFRA, and 0-2.5 µg/l or higher for SCC).

Statistical analyses were carried out using IBM SPSS, Statistics (version 25.0; IBM, Armonk, NY, USA), and R software (version 3.5.1).

Tumour marker measurement. Serum samples for measurement of serum tumour markers were collected within 1 month prior to bevacizumab plus chemotherapy. Serum levels of CEA were measured using a chemiluminescent method on a DXI 800i analyser (Beckman Coulter, Brea, CA, USA). Serum levels of CYFRA 21-1 were measured using an immunoradiometric titration method on a Stratec 300 analyser (Immunotech, Prague, Czech Republic). Serum levels of SCC were measured using a chemiluminescent method on an Architect i1000 analyser (Abbott Laboratories, Wiesbaden, Germany).

Table I. *Baseline patient characteristics.*

Parameter	n (%)
Gender	
Male	65 (61.9%)
Female	40 (38.1%)
Smoking status	
Non-smoker	22 (21.0%)
Former smoker	31 (29.5%)
Smoker	52 (49.5%)
ECOG PS	
0	4 (3.8%)
1	93 (88.6%)
2	8 (7.6%)
Line of therapy	
First	104 (99.1%)
Second	1 (1.0%)
Histology	
Adenocarcinoma	100 (95.2%)
Other	5 (4.8%)
Stage	
III	6 (5.8%)
IV	99 (94.3%)
Type of chemotherapy	
Carboplatin + paclitaxel	96 (91.4%)
Carboplatin + docetaxel	2 (1.9%)
Other	7 (6.7%)

ECOG PS: Eastern Cooperative Oncology Group performance status.

Results

Patient characteristics. A total of 105 patients were included in this retrospective analysis, including 65 males and 40 females, with a median age of 63 years. The baseline patient characteristics are summarized in Table I.

Overall response rate. There was no significant relationship between the ORR and the calculated oncomarkers. There was only somewhat of a trend in the case of CYFRA, with patients exceeding the norm characterized by more progressive disease. The results are summarized in Table II.

Univariate analysis of PFS and OS. There were no significant differences in OS or PFS in relation to serum CEA and SCC levels. We observed significantly better OS ($p=0.013$) and PFS ($p=0.021$) when CYFRA was within the norm. Patients with higher CYFRA values had poorer prognoses. The results are summarized in Table III. Kaplan–Meier curves for PFS and OS in relation to CYFRA are shown in Figures 1 and 2.

Multivariate Cox proportional hazards model. A Cox model was produced for demographic variables (age, gender, smoking status, ECOG PS) and for serum CYFRA level in predicting OS and PFS.

Table II. *Relationships of serum carcinoembryonic antigen (CEA), C-terminus of cytokeratin 19 (CYFRA), and squamous cell carcinoma antigen (SCC) levels with overall response rate.*

	CEA level, n (%)			CYFRA level, n (%)			SCC level, n (%)		
Response	Normal	Abnormal	*p*-Value	Normal	Abnormal	*p*-Value	Normal	Abnormal	*p*-Value
CR+PR	5 (25.0%)	30 (45.5%)	0.125	10 (41.7%)	24 (40.7%)	>0.999	23 (35.9%)	9 (50.0%)	0.290
CR+PR+SD	12 (60.0%)	51 (77.3%)	0.153	21 (87.5%)	40 (67.8%)	0.099	48 (75.0%)	11 (61.1%)	0.252

CR: Complete response; PR: partial response; SD: stable disease.

Table III. *Relationships of serum carcinoembryonic antigen (CEA), C-terminus of cytokeratin 19 (CYFRA), and squamous cell carcinoma antigen (SCC) levels with progression-free (PFS) and overall (OS) survival.*

	Median PFS (95% CI), months			Median OS (95% CI), months		
Parameter	Normal level	Abnormal level	*p*-Value	Normal level	Abnormal level	*p*-Value
CEA	6.1 (4.5-8.8)	5.8 (5.1-7.0)	0.515	12.9 (8.6-NA)	15.5 (11.7-20.1)	0.724
CYFRA	7.0 (5.7-11.5)	5.3 (4.5-6.5)	**0.021**	23.9 (16.8-NA)	11.7 (8.6-17.5)	**0.013**
SCC	6.0 (5.1-7.4)	6.1 (1.4-9.5)	0.754	15.5 (11.7-20.1)	11.7 (4.1-NA)	0.876

NA: Not achieved. Significant *p*-Values are shown in bold.

Only CYFRA was found to be significant as a predictor of OS. Patients with CYFRA higher than the norm had a 2.848 greater risk of death than did patients having CYFRA within normal limits. We observed no significant results in the Cox model for PFS. The results are summarized in Table IV.

Discussion

The data from the present retrospective analysis indicate for the first time a possible prognostic and theoretically predictive value for serum CYFRA in patients with NSCLC treated with bevacizumab. In contrast, no such value was demonstrated for CEA and SCC in relation to this treatment.

Earlier studies on the topic of oncomarkers as being possibly prognostic or predictive in patients treated with bevacizumab had been conducted in patients with colorectal cancer (CRC) (11-13). Andrade *et al*. showed in their case report a correlation between CEA and tumour response in patients with CRC (11). Prager *et al*. (12) and Holch *et al*. (13) then demonstrated in their studies the possible predictive potential of baseline CEA in patients with CRC treated with bevacizumab *versus* those treated with cetuximab. Because an association between CEA and response to bevacizumab had been suggested by the angiogenic potential of CEA in an *in vitro* model (14), the predictive potential of CEA in patients treated with bevacizumab was investigated in several NSCLC studies in (15-17). Duan *et al*. indicated a greater decrease in CEA during treatment when bevacizumab was added to chemotherapy (15). In our group of patients, unfortunately, the specific levels of oncomarkers during treatment were not available for most of the patients. Therefore, we cannot compare our findings in this regard. Du *et al*. then examined the level of CEA within effusions in patients with NSCLC treated with bevacizumab (16). As in our study, they concluded that CEA does not appear to be a specific marker for efficacy of bevacizumab treatment in NSCLC. Du *et al*. measured CEA in effusions, not, as in our study, in peripheral blood. The relationship of CEA in peripheral blood of patients with NSCLC treated with bevacizumab was also investigated by Zhang *et al*. (17). Similarly to our study, baseline CEA values were found not to be related to PFS under bevacizumab treatment. On the contrary, the development of these values proved to be a promising marker for predicting the subsequent response to bevacizumab treatment. Theirs was a small study of only 10 patients, however, so the required statistical strength of the study was not achieved in determining baseline values for response to treatment. Nevertheless, the overall observations regarding the relationship to baseline CEA are in contrast to the observations for CRC. Theoretically, this might be due to the different overall microenvironment of these tumours, as well as to the different chemotherapy used with bevacizumab. This may be suggested by the example from NSCLC, where the addition of paclitaxel *versus* gemcitabine to a platinum derivative and bevacizumab led to different results from treatment in clinical trials (2, 18). In addition, the model
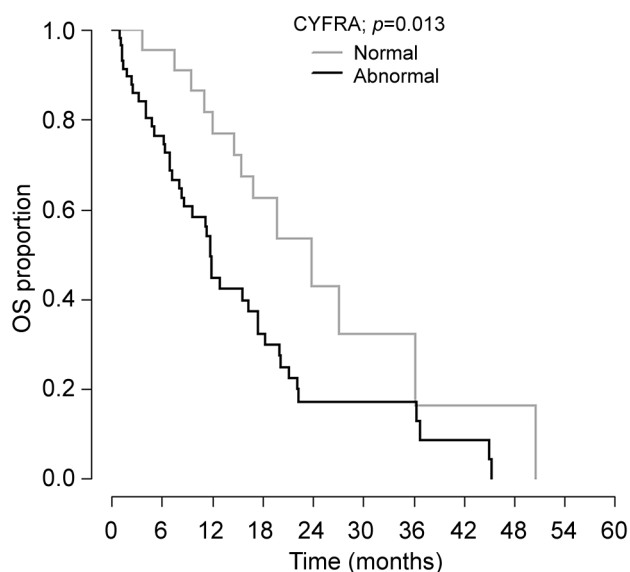
Figure 1. *Kaplan–Meir curves for overall survival (OS) in relation to the level of C-terminus of cytokeratin 19 (CYFRA) in serum.*
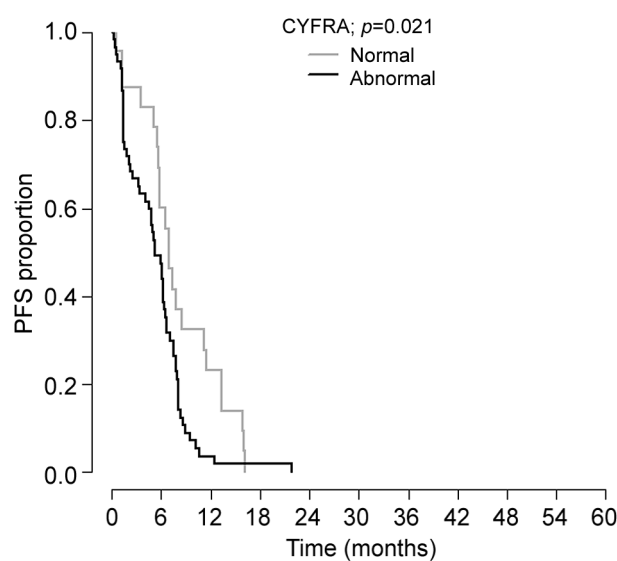

Figure 2. *Kaplan–Meir curves for progression-free survival (PFS) in relation to the level of C-terminus of cytokeratin 19 (CYFRA) in serum.*

Table IV. *Multivariate Cox proportional hazards model for overall (OS) and progression-free (PFS) survival.*

	OS		PFS	
Category	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Gender				
Male	Reference		Reference	
Female	1.277 (0.594-2.744	0.531	1.504 (0.787-2.876)	0.217
Age at diagnosis				
Increase by 1 year	0.982 (0.949-1.016)	0.297	1.002 (0.971-1.034)	0.889
Smoking status				
Non-smoker	Reference		Reference	
Former-smoker	0.600 (0.239-1.507)	0.277	0.662 (0.314-1.399)	0.280
Smoker	0.729 (0.313-1.700)	0.464	0.748 (0.385-1.451)	0.390
ECOG PS				
0	Reference		Reference	
1	0.790 (0.224-2.785	0.713	0.416 (0.125-1.387)	0.154
2	1.459 (0.344-6.193)	0.608	0.720 (0.171-3.042)	0.655
CYFRA				
Normal	Reference		Reference	
Abnormal	2.848 (1.254-6.465)	**0.012**	1.650 (0.850-3.205)	0.139

CI: Confidence intervaI; CYFRA: C-terminus of cytokeratin 19; ECOG PS: Eastern Cooperative Oncology Group performance status; HR: hazard ratio. Significant *p*-Values are shown in bold.

supporting the angiogenic effect of CEA *in vitro* was not an NSCLC model but rather a gastric cancer model (14).

We found only one study in the English-language literature examining the relationship between CYFRA levels and bevacizumab treatment in NSCLC (15). Duan *et al*. indicated a greater decrease in CYFRA during treatment when bevacizumab was added to chemotherapy (15). To the best of our knowledge, our work is the first to point to the CYFRA level as a prognostic marker for NSCLC treated with bevacizumab. However, a relationship between CYFRA and PFS was only confirmed in the univariate model and not in the Cox model. Similarly, the influence of CYFRA on

ORR, despite numerical differences, was only close to being statistically significant. With this in mind, we are of the opinion that prospective work with sufficient statistical power should be carried out.

The prognostic potential of CYFRA has also been supported by trials in lung adenocarcinomas treated with erlotinib or pemetrexed (8, 9, 19). On the contrary, however, it is notable that in a case of immunotherapy, Shirasu *et al*. published a trial with completely opposite results, showing that higher levels of CYFRA were associated with better PFS under nivolumab therapy (20). Due to there being a different mechanism of action at work in the case of immunotherapy and chemotherapy and because a different treatment was utilized, it is possible that CYFRA would show different results in the two cases. That study's authors had speculated that a higher number of mutations in patients with higher CYFRA levels could have played a role in this regard (20).

SCC is usually taken as a marker for squamous NSCLC, but above-limit SCC values may also be observed in some patients with lung adenocarcinomas (21). In a study of patients who underwent surgery for adenocarcinoma, moreover, higher levels led to a poorer prognosis (22). A theoretical explanation may be the presence of squamous elements in these patients with pulmonary adenocarcinomas (23). Because for safety reasons bevacizumab is not used in patients with squamous NSCLC (3), we wondered if a higher SCC level would lead to a poorer prognosis for bevacizumab-treated patients. We did not confirm this hypothesis in our study. To our knowledge, there is no other similar study dealing with this topic.

The present study has several limitations. Firstly, this was a retrospective study that may have been biased with regard to patient selection. Secondly, PFS was not confirmed by an independent board. Finally, some data were incomplete and therefore some analyses lacked sufficient statistical power. The present report should thus be regarded as exploratory and the results should be verified in a larger prospective study.

Conclusion

In our retrospective study, we pointed out the possibility of using CYFRA as a prognostic marker in patients with NSCLC treated with chemotherapy plus bevacizumab. This result, as well as the possible predictive use of CYFRA, should be verified in a larger prospective study.

Conflicts of Interest

In connection with this article, the Authors declare they have in the past provided consulting services to Roche.

Authors' Contributions

MS and JB conceived the presented idea. MS, JB, GK, MP, MB, ZT, JV, and OT conceived and planned the study and collected the data. MS and JB analysed the data. MS wrote the article with support from JB. OP helped supervise the project.

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