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The Applicability of a Predictive Index for Second- and Third-Line Treatment of Unselected Non-Small-Cell Lung Cancer Patients

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Key Words
Predictive index · Erlotinib · Non-small-cell lung cancer · *EGFR* gene mutation

Abstract
Background: Tyrosine kinase inhibitors of EGFR (TKI-EGFR) induced response in only 10% of Caucasian non-small-cell lung cancer patients in second- or third-line treatment. Independent predictive factors for qualification to TKI-EGFR treatment have not been assessed. In 2008, a prognostic index was reported for patients treated with erlotinib in the BR.21 trial, but its application for real, unselected patients is limited. ***Objectives:*** Based on clinical and molecular factors of patients treated with erlotinib, we tried to create a predictive index which could be applied in real treatment practice. ***Methods:*** In a Cox regression model, we established 6 factors which affected overall survival for erlotinib treatment: performance status, erlotinib-induced rash, time from diagnosis to treatment, gender, weight loss and LDH level. We analyzed the risk factors of early progression and survival shorter than 6 months. In addition we included: time from first-line chemotherapy to erlotinib treatment, smoking status, mutation status in *EGFR* and anemia. ***Results:*** Our model consisted of 10 factors that were assigned points according to HR or χ^2 and p value. The score was used to separate patients into 4 risk categories of unfavorable disease course based on 10th, 50th and 90th percentiles: low risk (I), intermediate low risk (II), intermediate high risk (III) and high risk (IV). Survival probability was significantly higher for group I, intermediate for groups II and III, and significantly lower for group IV ($\chi^2 = 49.5$, $p < 0.0001$). Based on the previously reported index we could not qualify our patients for the low risk group. ***Conclusions:*** Our model could be useful for qualification for erlotinib treatment of patients with numerous adverse factors and limited access to genetic examination.

Introduction

The clinical response for the second and third lines of treatment with tyrosine kinase inhibitors of EGFR (TKI-EGFR) occurs in less than 10% of Caucasian, unselected

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non-small-cell lung cancer (NSCLC) patients [1, 2]. The independent predictive factors according to which the patients could be classified for erlotinib therapy have not yet been assessed. However, in 2008 Florescu et al. [3] described a clinical prognostic index for patients treated with erlotinib. It should be mentioned that the patients included in the Florescu study were precisely selected according to the BR.21 guidelines. The scale created by Florescu et al. [3] is based on the analysis of 10 clinical and molecular factors which had an impact on the overall survival of the patients. The following parameters were classified into groups of adverse factors: performance status (PS) 2–3, current or former smoking, weight loss >5% prior to therapy, anemia at the beginning of treatment, short time from diagnosis to erlotinib treatment, progression during prior chemotherapy, disomy or low polysomy of *EGFR* gene in fluorescence in situ hybridization (FISH) examination or no FISH results, ethnicity other than Asian and erlotinib in third-line treatment. The particular parameters were scored and 4 groups of different erlotinib therapy outcome were created to describe the risk for therapy failure: group 1 of low risk: score less than 18 points; group 2 of intermediate low risk: 18–27 points; group 3 of intermediate high risk: 28–38 points; group 4 of high risk: score higher than 38 points. Then, Florescu et al. [3] showed that the scale is rather of prognostic than predictive value, except for the qualification of the first group.

Furthermore, the practical application of the prognostic-predictive index created by Florescu et al. [3] encountered some difficulties. The real patients, unselected for clinical trials, are charged with more adverse clinical factors, and genetic examination is still not available in many clinical centers. There is a possibility that ethnic difference may influence the role of Florescu et al.'s index in a Caucasian population. Florescu et al.'s report also included Asian patients in the examined population. It is common knowledge that mutation in exon 18–21 of *EGFR* gene occurs more frequently (70–80%) in adenocarcinoma tumors among Asian, female, nonsmoking patients than in squamous cell carcinoma among Caucasian, male, heavy smoking patients (less than 7%). The presence of *EGFR* mutation, especially deletion in exon 19 and point mutation L858R in exon 21, have been correlated with clinical benefit to TKI-EGFR treatment [4–9].

According to Hirsch and Bunn [10], 'the prognostic and predictive value of various combinations of biomarkers might need to be studied, not just the value of an individual biomarker'.

Patients and Methods

Patient Characteristics

The study group included 73 patients (51 male and 22 female; mean age 61.64 ± 8.19 years; median 62 years) with advanced stages (IIIB, IV) of NSCLC. We had applied an age cutoff of 65 years during our index creation. For the qualification of our patients according to Florescu et al.'s scale, we used a cutoff of 60 years.

According to the smoking status, the patients were categorized as heavy, current smoker (n = 56) and light, current smoker (n = 5). Medium value of pack-years was estimated as 32.93 ± 26.16 (maximum value was 132 pack-years). Five patients had never been smoking. In our group, only 2 smoking patients were former smokers. However, when we used Florescu et al.' s scale we divided our patients into current and former smokers.

In all patients, the platinum-based doublet chemotherapy was the first line of treatment. Bevacizumab was implemented in 7 patients. The second-line therapy was composed of docetaxel (n = 15) or gemcitabine monotherapy (n = 1). Therefore, erlotinib as a second or third line of treatment was applied in 57 and 16 patients, respectively. The patients with short observation and short period of erlotinib treatment (<2 weeks) and very bad PS (4) were excluded from analysis. The patients who died suffering from disease other than lung cancer were also excluded. The median time from diagnosis to erlotinib treatment was 8 months (13.49 ± 14.78; range: 2–82). Median time of erlotinib treatment was 9 weeks (range: 2–95). The follow-up range for patients treated with erlotinib was September 2007 till March 2010.

In addition, the agreement of the Ethics Committee of the Medical University of Lublin was obtained.

Immunohistochemistry and FISH Analysis

Immunohistochemistry and FISH examination were performed for 23 patients (31.5%), but in 7 cases we could not obtain clear FISH results.

Expression of EGFR protein was estimated using immunohistochemistry method with monoclonal antibody according to diagnostic protocol (EGFRpharmDx; DAKO). The number of *EGFR* gene copies were investigated by FISH using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis) and the Paraffin Pretreatment Kit (Abbott Molecular Inc.) according to the enclosed protocols. The FISH analysis was performed independently by two observers in at least 100 nonoverlapping nuclei. According to the frequency of the tumor cells with specific number of the *EGFR* gene and chromosome 7 copies, the patients were defined as described previously [1, 8, 11, 12].

Mutation Analysis in Exon 19 and 21 in EGFR *Gene*

Mutation analysis was performed for 24 patients (32.9%). We used PCR and PCR-RFLP techniques as previously described and fluorescently (CY5) labeled primers to detect short in-frame deletion in exon 19 and point mutation L858R in exon 21 of *EGFR* gene, respectively [13]. Moreover, we used ARMS-PCR technique and the fluorescently labeled primers modified by us to confirm the absence of mutation in exon 21 (methodology data provided on request).

The analysis was performed in ALFWin Fragment Analyzer with Allele-Fragment Analysis program. The PCR fluorescently labeled and Sau96I-digested fluorescently labeled products were

denatured at 95°C for 3 min, cooled on ice and subjected to polyacrylamide gel in ALFWin Analyzer. DNA isolated from H1650 and H1975 tumor cell lines served as a positive control for the presence of mutations in exon 19 and 21, correspondingly. To estimate the sensitivity of the applied technique, the serial dilutions of DNA from lung cancer cell lines H1650 or H1975 with normal DNA isolated from blood leukocytes of healthy donor were compared.

Statistical Analysis

Since the presented study constitutes the discussion with the report presented previously, the statistical analysis was based on the methods described by Florescu et al. [3]. However, our research was not controlled by placebo. In short, χ^2 test was used to compare the characteristics of the patient groups divided according to the significance of selected clinical and molecular factors. The Cox regression model with stepwise selection procedures by minimum AIC was used to establish a novel predictive model for erlotinib-treated patients. Six variables gave a minimum AIC to the Cox model (table 1). Additionally, we had chosen another 4 variables affecting the disease progression and survival shorter than 6 months based on the χ^2 test (tables 2, 3). Those selected variables were assigned points based on their significance level (estimated coefficient in the predictive model and in χ^2 test; table 4), then they were applied to establish 4 categories of risk (table 5) according to the 10th, 50th and 90th percentile of the index score. The Kaplan Meier method was used for the comparison of survival probability between the groups of different risk of erlotinib therapy failure. The Mann-Whitney U test was used for assessing whether two independent samples of observation come from the same distribution.

Additionally, we divided the patients into groups with different risk of erlotinib treatment failure according to Florescu et al.'s index (table 6).

Results

Estimation of Early Progression in Correlation with Clinical and Molecular Factors

Disease control, marked as stable disease (SD), partial response (PR) and early progression during the first 2 months of therapy was noted in 23 (31.5%) and 50 (68.5%) patients, respectively. PR was noted in 9 patients (12.3%), while complete response was not observed in our group. The median progression-free survival of patients with disease control was 7 months (mean ± standard deviation: 8.41 ± 4.69 months; range: 3–25).

The factors significantly correlating with disease control were: PS 0–1 ($p < 0.00001$), female gender ($p < 0.05$), never or light smoking ($p < 0.005$), rash ($p < 0.00001$), elongated time from first-line chemotherapy ($p < 0.005$) and *EGFR* gene mutation presence ($p < 0.05$) (table 2). These factors had predictive value.

Table 1. Factors that significantly affected overall survival of erlotinib-treated patients

Factors	Coefficient β	p	Hazard ratio
Male	0.7831	<0.05	2.19 (1.15–4.16)
PS 2–3	2.5228	<0.0001	12.46 (5.37–28.94)
Weight loss >5% before treatment	0.816	<0.01	2.26 (1.27–4.04)
Time from diagnosis to treatment >12 months	1.1104	<0.005	3.03 (1.52–6.07)
Lack of rash	1.5211	<0.0001	4.58 (2.14–9.79)
LDH elevation	0.8496	0.052	2.34 (1.0–5.48)

Figures in parentheses are 95% CI.
Overall model fit: $\chi^2 = 80.21$, $p < 0.0001$.

Assessment of Survival Shorter than 6 Months in Correlation with Clinical and Molecular Factors

Median time of overall survival was 5 months (mean: 6.75 ± 5.71 months; range: 1–28). Survival longer than 6 months from the beginning of treatment was observed in 32 patients (43.8%). Survival longer than 6 months was significantly associated with: PS 0–1 ($p < 0.00001$), never or light smoking ($p < 0.01$), long period since diagnosis ($p < 0.01$) and long period from first-line chemotherapy ($p < 0.05$). Interestingly, the longer survival of patients was slightly correlated with: female gender ($p = 0.089$), response to first-line chemotherapy ($p = 0.072$) and *EGFR* gene mutation presence ($p = 0.053$) (table 3). These factors had rather prognostic than predictive value.

Molecular Markers and Treatment Response Rates

In our study we found that EGFR protein expression and *EGFR* gene copy number had no significant influence on early progression and survival shorter than 6 months. However, the presence of *EGFR* gene mutation importantly affected the disease control as well as survival longer than 6 months of erlotinib-treated patients (tables 2, 3). In examined tissue sample we found 3 mutations in exon 19 and 2 mutations in exon 21 of *EGFR* gene.

Among the patients with FISH examination, 75% of patients with high copy number and 87.5% with low copy number of *EGFR* gene were classified as early progression ($\chi^2 = 0.41$, $p = 0.52$). Low number of copies was observed slightly more frequently in squamous cell carcinoma than in other histopathological types of NSCLC (χ^2 =

Table 2. Influence of clinical and molecular factors on early progression risk during the first 2 months of erlotinib treatment

Factor		Patients	Early PD	Disease control	p	χ^2
Whole group		73	50	23		
Age	<65 years	25	17 (68%)	8 (32%)	0.948	0
	>65 years	48	33 (68.75%)	15 (31.25%)		
Gender	Male	51	39 (76.5%)	12 (23.5%)	<0.05	4.99
	Female	22	11 (50%)	11 (50%)		
Smoking status	>10 pack-years (heavy smoker)	56	44 (78.6%)	12 (21.4%)	<0.005	12.09
	<10 pack-years (light smoker)	5	1 (20%)	4 (80%)		
	Never smoker	12	5 (41.7%)	7 (58.3%)		
PS	0/1	30	12 (40%)	18 (60%)	<0.0001	19.16
	2/3	43	38 (88.4%)	5 (11.6%)		
Histopathology	Adenocarcinoma	45	31 (68.9%)	14 (31.1%)	0.93	0.01
	Other types of NSCLC	28	19 (68%)	9 (32%)		
Response to first-line treatment	Complete response, PR, SD	49	31 (63.3%)	18 (36.7%)	0.169	1.89
	Progressive disease	24	19 (79.2%)	5 (20.8%)		
Prior regimens	Second-line treatment	59	41 (69.5%)	18 (30.5%)	0.706	0.14
	Third- or fourth-line treatment	14	9 (64.3%)	5 (35.7%)		
Time from diagnosis	<12 months	49	38 (77.5%)	11 (22.5%)	0.173	5.67
	>12 months	24	12 (50%)	12 (50%)		
Weight loss during 3 months	<5%	46	30 (65.2%)	16 (34.8%)	0.432	0.62
	>5%	27	20 (74.1%)	7 (25.9%)		
Anemia	Yes	54	40 (74.1%)	14 (25.9%)	0.0836	2.99
	No	19	10 (52.6%)	9 (47.4%)		
Rash	Yes	26	10 (38.5%)	16 (61.5%)	<0.0001	16.88
	No	47	40 (85.1%)	7 (14.9%)		
Time from first-line treatment	<12 months	58	43 (74.1%)	15 (25.9%)	0.00412	4.17
	>12 months	15	7 (46.7%)	8 (53.3%)		
Disease stage	III B	21	12 (57.1%)	9 (42.9%)	0.1846	1.76
	IV	52	38 (73.1%)	14 (26.9%)		
Prior radiotherapy	Yes	23	13 (56.5%)	10 (43.5%)	0.1354	2.23
	No	50	37 (74%)	13 (26%)		
Prior surgical treatment	Yes	12	7 (58.3%)	5 (41.7%)	0.4072	0.69
	No	61	43 (70.5%)	18 (29.5%)		
Serum LDH level	High	14	9 (64.3%)	5 (35.7%)	0.461	1.549
	Normal	16	13 (81.2%)	3 (18.8%)		
	Unknown	43	28 (65.1%)	15 (34.9%)		
EGFR protein expression	EGFR positive	16	11 (68.75%)	5 (31.25%)	0.5751	1.106
	EGFR negative	7	6 (85.7%)	1 (14.3%)		
	Unknown	50	33 (66%)	17 (34%)		
EGFR gene copy number	High copy number	8	6 (75%)	2 (25%)	0.3995	1.835
	Low copy number	8	7 (87.5%)	1 (12.5%)		
	Unknown/failed	57	37 (64.9%)	20 (35.1%)		
EGFR gene mutation status	Mutation in exon 19 or 21	5	1 (20%)	4 (80%)	<0.05	6.43
	None	19	15 (78.9%)	4 (21.1%)		
	Unknown	49	34 (69.4%)	15 (30.6%)		

Table 3. Influence of clinical and molecular factors on 6-month survival of erlotinib-treated patients

Factor		Patients	Survival <6 month	Survival >6 month	p	χ^2
Whole group		73	44 (60.3%)	29 (39.7%)		
Age	<65 years	48	31 (64.6%)	17 (35.4%)	0.297	1.09
	>65 years	25	13 (52%)	12 (48%)		
Gender	Male	51	34 (66.7%)	17 (33.3%)	0.088	2.89
	Female	22	10 (45.45%)	12 (54.55%)		
Smoking status	>10 pack-years (heavy smoker)	56	38 (67.85%)	18 (32.15%)	<0.01	9.46
	<10 pack-years (light smoker)	5	0	5 (100%)		
	Never smoker	12	6 (50%)	6 (50%)		
PS	0/1	30	6 (20%)	24 (80%)	<0.00001	34.5
	2/3	43	38 (88.4%)	5 (11.6%)		
Histopathology	Adenocarcinoma	45	27 (60%)	18 (40%)	0.9516	0
	Other types of NSCLC	28	17 (60.7%)	11 (39.3%)		
Response to first-line treatment	Complete response, PR, SD	49	26 (53.1%)	23 (46.9%)	0.0719	3.24
	Progressive disease	24	18 (75%)	6 (25%)		
Prior regimens	Second-line treatment	59	37 (62.7%)	22 (37.3%)	0.382	0.76
	Third- or fourth-line treatment	14	7 (50%)	7 (50%)		
Time from diagnosis	<12 months	49	35 (71.4%)	14 (28.6%)	0.005	7.74
	>12 months	24	9 (37.5%)	15 (62.5%)		
Weight loss during 3 months	<5%	46	26 (56.5%)	20 (43.5%)	0.3925	0.73
	>5%	27	18 (66.7%)	9 (33.3%)		
Anemia	Yes	54	35 (64.8%)	19 (35.2%)	0.181	1.79
	No	19	9 (47.4%)	10 (52.6%)		
Rash	Yes	26	9 (34.6%)	17 (65.4%)	<0.005	9.5
	No	47	35 (74.5%)	12 (25.5%)		
Time from first-line treatment	<12 months	15	5 (33.3%)	10 (66.6%)	<0.05	5.72
	>12 months	58	39 (67.24%)	19 (32.76%)		
Disease stage	III B	21	10 (47.6%)	11 (52.4%)	0.16	1.97
	IV	52	34 (65.4%)	18 (34.6%)		
Prior radiotherapy	Yes	23	11 (47.8%)	12 (52.2%)	0.141	2.17
	No	50	33 (66%)	17 (34%)		
Prior surgical treatment	Yes	12	6 (50%)	6 (50%)	0.426	0.63
	No	61	38 (62.3%)	23 (37.7%)		
Serum LDH level	High	14	10 (71.4%)	4 (28.6%)	0.361	2.034
	Normal	16	11 (68.7%)	5 (31.3%)		
	Unknown	43	23 (53.5%)	20 (46.5%)		
EGFR protein expression	EGFR positive	16	11 (68.75%)	5 (31.25%)	0.542	1.225
	EGFR negative	7	5 (71.4%)	2 (28.6%)		
	Unknown	50	28 (56%)	22 (44%)		
EGFR gene copy number	High copy number	8	6 (75%)	2 (25%)	0.395	1.856
	Low copy number	8	6 (75%)	2 (25%)		
	Unknown/failed	57	32 (56.1%)	25 (43.9%)		
EGFR gene mutation status	Mutation in exon 19 or 21	5	1 (20%)	4 (80%)	0.053	5.88
	None	19	14 (73.7%)	5 (26.3%)		
	Unknown	49	35 (71.4%)	14 (28.6%)		

Table 4. Scoring of prognostic and predictive factors in the calculation of prognostic index for erlotinib-treated patients

Factors	0 points	2 points	4 points	6 points	8 points	10 points
Performance status (ECOG/WHO)	0–1					2–3
Rash at the beginning of treatment	yes				no	
Time from diagnosis	>12 months			<12 months		
Weight loss	<5%		>5%			
Gender	female	male				
LDH level	normal/unknown	increased				
Time from first-line chemotherapy	<12 months		>12 months			
Smoking status	never/light			heavy		
EGFR mutation	presence	unknown	absence			
Anemia	absence	presence				

Table 5. Risk category of prognostic index for erlotinib-treated patients

Risk category	Definition points	Patients	Overall survival[a], months	p	χ^2
Low risk	0–16	10	19; 12 ± 7.3 (4–28)	<0.0001	49.466
Intermediate low risk	17–32	32	9; 8.8 ± 5.4 (1–24)		
Intermediate high risk	33–44	26	3.25; 3.15 ± 1.7 (0.5–6)		
High risk	>44	5	1.5; 1.8 ± 1.15 (0.5–3)		

[a] Median; mean ± standard deviation. Ranges are given in parentheses.

Table 6. Risk category according to Florescu et al.'s prognostic index for our erlotinib-treated patients

Risk category	Definition points	Patients	Overall survival[a], months	p	χ^2
Low risk	<18	0		<0.0001	35.404
Intermediate low risk	18–27	20	19; 11 ± 6.5 (3.5–28)		
Intermediate high risk	28–38	41	5; 6 ± 4.7 (0.5–21)		
High risk	>38	12	1.75; 2.25 ± 1.5 (0.5–5.5)		

[a] Median; mean ± standard deviation. Ranges are given in parentheses.

1.61, p = 0.2). Seven FISH-positive and 4 FISH-negative tumors simultaneously expressed EGFR protein.

Among the patients with mutation examination, 4 with PR and 1 with progressive disease (woman with brain and bone metastasis, 30 pack-years, PS = 3) were found. Objective response, disease control and survival longer than 6 months were observed significantly more frequently in patients with mutation (χ^2 = 18.24, p < 0.0001, χ^2 = 6.19, p < 0.05 and χ^2 = 4.87, p < 0.05, respectively). Among the group of patients without mutation (n = 19), 4 SD and 15 progressive disease were noted. Time for treatment failure was 16 months for 1 nonsmoking woman with adenocarcinoma, 8 months for 1 man with giant cell carcinoma (31 pack-years), 9 months for 1 man with adenocarcinoma (10 pack-years) and 6 months for 1 man with adenocarcinoma (20 pack-years). Mutation in

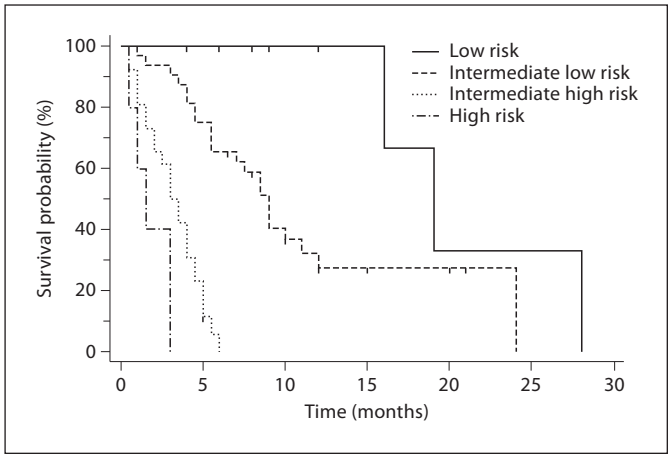

Fig. 1. Survival probability of 4 groups of erlotinib therapy failure risk scored by the presented index.

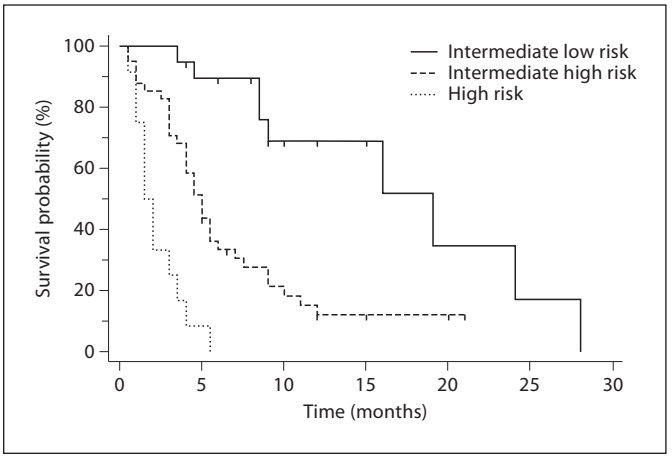

Fig. 2. Survival probability of 3 groups of erlotinib therapy failure risk scored by Florescu et al.'s index.

exon 19 or 21 was not found in patients with squamous cell carcinoma. Additionally, no correlation between mutation occurrence and gender as well as smoking history was observed.

Predictive Index

Based on the Cox regression model, we established 6 predictive factors of overall survival for erlotinib-treated patients: PS, rash at the beginning of treatment, time from diagnosis to treatment, gender, weight loss >5% and LDH level (table 1). Moreover, we analyzed high risk factors of early progression and survival shorter than 6 months from the beginning of erlotinib treatment. We additionally included: time from first chemotherapy to erlotinib treatment, smoking status, *EGFR* gene mutation status and anemia (tables 2, 3). Finally, our model consisted of 10 factors pointed according to HR or χ^2 value and their level of significance (table 4). The score was used to separate patients into 4 risk categories of unfavorable disease course: low risk (group I), intermediate low risk (group II), intermediate high risk (group III) and high risk (group IV) (table 5).

Patients with PR were classified into the low risk (n = 4) and intermediate low risk groups (n = 5), while patients with SD were in the low risk (n = 4) and intermediate low risk groups (n = 10). There were no patients with disease control within the intermediate high risk and high risk groups.

The probability of survival was significantly higher for the first group, intermediate for the second and third groups and significantly lower for the fourth group (χ^2 = 49.5, $p < 0.0001$; fig. 1). Of the patients, 13.7% were qualified for the first group and had obviously benefited from erlotinib therapy (median survival = 19 months). For the second and third groups, 43.8 and 35.6% of patients were qualified, respectively. The intermediate low risk group had significantly longer overall survival than the intermediate high risk group (median 9 vs. 3.25 months; $p < 0.00001$). Overall survival did not significantly differ between the intermediate high and high risk groups ($p = 0.082$). The patients from those groups did not seem to benefit from erlotinib therapy.

Based on Florescu et al.'s index, we could not qualify our patients for the low risk group. Our patients had numerous clinical factors, such as smoking history, PS 2 and short period from diagnosis to treatment. More than half of the patients with disease control (12, including 5 with PR and 7 with SD) were qualified for the intermediate high risk group, while the remaining were put into the intermediate low risk group. Patients from the second and third groups seem to benefit from erlotinib-based therapy.

Probability of survival significantly correlated with risk of therapy failure, calculated by Florescu et al.'s index ($\chi^2 = 35.4036$, $p < 0.0001$; fig. 2). Median survival of the intermediate low risk group was significantly higher than that of the intermediate high ($p < 0.0005$) and high risk ($p < 0.00001$) groups. What is more, the intermediate high risk group had significantly higher median overall survival when compared with the high risk ($p < 0.005$) group. The patients from the high risk group had no benefit of survival (table 6).

Based on these results, our index could be applied for both qualification and discrimination of the patients for erlotinib therapy, while the Florescu index could be useful only for therapy discrimination.

Discussion

Less than 10% of patients with advanced stages of NSCLC may achieve objective response with second-line treatment composed of docetaxel, pemetrexed or erlotinib. Despite an overall response rate, patient survival had significantly improved after second-line treatment. Furthermore, the patients who received second-line chemotherapy lived 2–3 months longer than those receiving only the best supportive care [2, 14]. Independent predictive prognostic factors for overall survival for patients treated with second-line chemotherapy were: female gender, primary and locally advanced NSCLC, objective responses for first-line chemotherapy, and PS 0–1 [15]. It has been demonstrated that pemetrexed and erlotinib are more beneficial especially in adenocarcinoma-bearing patients [2–6, 14, 16]. Moreover, the erlotinib-based therapy could be effective even for patients with bad PS and after failure of second-line treatment based on docetaxel or pemetrexed [2].

In 2008, Florescu et al. [3] published one of the most important reports concerning the possibility to create a clinical prognostic index. The main objective of their study was to find any additional factors, besides those of prognostic attributes of second-line treatment, particularly affecting the outcome of erlotinib-treated patients (such as smoking status, ethnicity and FISH result). The predictive value of those factors was also established previously [1, 8, 17]. Florescu et al. [3] had defined 4 groups within the analyzed population that had shown distinctive survival outcomes. They suggested that the patients classified into the low risk group had the greatest benefit from erlotinib-based therapy. On the other hand, the clinical prognostic index established by Florescu et al. is of questionable value for unselected NSCLC patients of homogenous Caucasian ethnicity. Furthermore, it should be considered that Florescu et al.'s study population consists of precisely selected patients according to the BR.21 trial guidelines. The unselected patients are loaded with numerous predictive factors. Based on Florescu et al.'s index, we could not single out the low risk group within our patients. Therefore, this index had lost predictive value for our patients. It should be kept in mind that our study is not placebo controlled and it only considers a small population, therefore its clinical value is limited.

The predictive role of clinical factors in erlotinib-treated patients is controversial. The influence of factors such as time from diagnosis to erlotinib therapy, progression-free survival after first-line chemotherapy as well as response to previous therapies on the efficacy of erlotinib-based therapy was estimated by multiparameter and single-parameter analysis. Time from diagnosis to erlotinib therapy depends on other factors (for example, dynamics of tumor cell proliferation) than response to chemotherapy (for example, expression of DNA-repair enzymes in platine-dublets therapy or *MDR1* polymorphism in docetaxel-cisplatin therapy) [18, 19]. Therefore, in a Cox regression model, time from diagnosis to erlotinib therapy has a significant value for overall survival, but the other two factors do not. We confirmed that time from diagnosis to erlotinib therapy is rather a prognostic factor, while response to chemotherapy is a predictive one. On the other hand, progression-free survival and response to first-line chemotherapy have impact on erlotinib efficacy. In this respect, the poor PS and weight loss are rather prognostic than predictive factors.

Searching for predictive factors in our study, we observed that women and nonsmokers are responding more frequently to erlotinib than men, which is in keeping with previous reports [2]. Moreover, the low number of *EGFR* gene copies did not affect the efficacy of erlotinib-based therapy, as opposed to *EGFR* mutation in exon 19 or 21. In our model the presence of *EGFR* gene mutation had an important effect on therapy efficiency as well as on 6-month survival of erlotinib-treated patients. The influence of molecular factors on the outcome of erlotinib-treated patients was also confirmed by clinical trials: TRUST and SATURN [18, 20, 21]. Similarly to our study, the retrospective TRUST clinical trial was an open-label single-arm study not controlled by placebo, while SATURN was one of the largest prospective studies concerning molecular markers in TKI-EGFR therapy of NSCLC. These trials demonstrated the favorable predictive role of *EGFR* gene mutation in tumor cells (higher frequency of objective response, prolongation of progression-free survival and overall survival) [7]. Moreover, the SATURN trial proved that FISH examination does not have predictive value in patients with *EGFR* wild-type gene. Owing to these results, FISH examination is just about to lose its application to patient qualification to TKI-EGFR treatment.

In our group, the *EGFR* gene mutation occurs mostly among smoking patients. In contrast, meta-analyses conducted by Toyooka et al. [22] and Mitsudomi and Yatabe [5] have shown the predominant occurrence of *EGFR* mutation in exon 19 and 21 in nonsmoking women. In addition, the predictive role of rash is still controversial because the essential condition of rash appearance is the treatment beginning [2].

Our predictive and prognostic score was able to divide the patients into 4 groups of different erlotinib-therapy outcome. Patients classified into the low risk group of therapy failure had significantly the longest overall survival. Moreover, there are no contraindications to enclose the patients of intermediate low risk for erlotinib-based therapy. On the contrary, the patients of the intermediate high risk and high risk groups are unlikely to benefit at all; furthermore, these patients should not be treated with erlotinib. The estimation of *EGFR* gene mutation could be very helpful to qualify the patients from the intermediate low risk group and some patients from the intermediate high risk group to erlotinib therapy.

As previously remarked by Florescu et al. [3] our predictive prognostic index based on molecular and clinical factors requires prospective validation in a placebo-controlled study to confirm that it is both of prognostic and predictive value. Lack of a placebo arm limits the usefulness of our results to predict the response or control of disease. However, it could be of practical application in a situation where the approach to molecular tests is limited or their sensitivity is not sufficient (for example, suspicion of false negative results) due to heterogenous and degraded materials (tumor tissue). If the genetic examinations are reliable, molecular results are more useful for TKI-EGFR treatment qualification than multifactor indexes. Especially if *EGFR* mutation is present in tumor cells, we should not hesitate to apply this type of treatment even in very advanced stages of the disease if PS is adequate. Finally, the presented index could be applied for qualification to or discrimination from erlotinib therapy. Among our group of patients, Florescu et al.'s index could be useful only for the discrimination from erlotinib therapy. Moreover, our index could be applied in a homogenous Caucasian, heavy and current smoking population.

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