# Predictive and Prognostic Significance of Sodium Levels in Patients with NSCLC Treated by Erlotinib

MARTIN SVATON[1], ONDREJ FIALA[2], MILOS PESEK[1], FRANTISEK BRUHA[1], PETR MUKENSNABL[3], JAROSLAV RACEK[4], MAREK MINARIK[5] and ZDENEK BORTLICEK[6]

*Departments of [1]Pneumology and Phthisiology, [2]Oncology and Radiotherapy, [3]Pathology, [4]Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University in Prague, Prague, Czech Republic; [5]Centre for Applied Genomics of Solid Tumours (CEGES), Genomac Research Institute, Prague, Czech Republic; [6]Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic*

**Abstract.** *Background: Hyponatremia is a well-known phenomenon in cancer patients. The aim of our retrospective study was to assess the relationship of natremia levels to predict treatment with erlotinib and also to assess the prognosis of patients with hyponatremia. Patients and Metods: We analyzed data of 544 patients with advanced-stage non-small cell lung cancer (NSCLC) treated with erlotinib. Results: Hyponatremia was measured in 21.5 % of patients before treatment with erlotinib. We found a significant increase in the effectiveness of treatment with erlotinib in patients with normal levels of sodium to hyponatremic patients. Progression-free survival (PFS) and overall survival (OS) were also significantly higher in patients with normal natremia. Multivariate Cox model analysis demonstrated that natremia was an independent factor for PFS and OS. Conslusion: We reported hyponatremia not only as a prognostic marker in NSCLC patients but also as predictive marker of erlotinib treatment efficacy, being an independent factor at the present large retrospective study. Its possible effect in clinical practice is bigger thanks the simple possibility of testing of hyponatremia and the low cost of this biomarker.*

Lung cancer is one of the leading causes of cancer-related mortality throughout the world (1). Non-small cell lung cancer (NSCLC) represents a predominant histological type of lung cancer (2). Most patients are initially diagnosed with advanced-stage disease (*i.e.* locally-advanced or metastatic stage) (4), which correlates with poor prognosis and a low 5-year overall survival (OS) (3). Several new effective agents, including erlotinib, have been recently approved for the treatment of NSCLC patients. Erlotinib is a low-molecular weight inhibitor targeting epidermal growth factor receptor (EGFR) tyrosine kinase. The efficacy and safety of erlotinib in patients with advanced NSCLC has been demonstrated in various phase III clinical trials (6, 7). In the present study, we aimed at predictive and prognostic significance of pre-treatment sodium levels in patients with advanced-stage NSCLC treated with erlotinib.

*Correspondence to:* Martin Svaton, MUDr, Department of Pneumology and Phthisiology, Faculty of Medicine in Pilsen, Charles University in Prague, University Hospital Pilsen, Edvarda Benese 13, CZ-305 99 Pilsen, Czech Republic. Tel: +420 728129259, e-mail: svatonm@gmail.com

*Key Words:* Hyponatremia, NSCLC, PFS, OS, prognosis, predictive marker.

## Patients and Methods

*Patients' characteristics*. Totally, 544 patients were included in the study. The median age was 64 years (range, 28-84 years). There were 343 (63.1%) male patients and 201 (36.9%) female patients; 254 (46.7%) patients were current smokers, 189 (34.7%) patients former smokers and 101 (18.6%) patients never smokers; 255 (46.9%) patients suffered from adenocarcinoma, 260 (47.8%) patients suffered from squamous-cell cancer and 29 (5.3%) patients had not otherwise specified NSCLC; 430 (79.0%) patients were found in stage IV disease, 114 (21%) patients in stage IIIB disease, 304 (55.9%) patients had ECOG PS 0 or 1, 240 (44.1%) patients had PS 2 or 3. In total, 314 (57.8%) patients were tested for activating *EGFR* mutation, 280 (51.5%) of them were wild-type *EGFR* and 34 (6.3%) were *EGFR* mutation-positive. The baseline patients' characteristics are summarized in Table I.

*Study design and treatment*. We retrospectively analyzed clinical and laboratory data of 544 patients with cytologically or histologically confirmed advanced-stage (stage IIIB or IV) NSCLC treated with erlotinib between 2006 and 2013 at the Department of Pneumology and Phthisiology, University Hospital in Pilsen. The baseline clinical characteristics of patients are summarised in Table I. Totally, 314 patients were tested for activating *EGFR* mutation (exon 19 deletion and point mutation in exon 21, L858R); 34 (10.8%) of them were *EGFR* mutation positive and 280 (89.2%) harboured wild-type *EGFR* gene. Erlotinib was administered orally in a standard dose of 150 mg

Table I. *Baseline clinical characteristics.*

| | | Total (n=544) |
|---|---|---|
| Gender, n (%) | Males | 343 (63.1) |
| | Females | 201 (36.9) |
| Age (years) | Median, (min-max) | 64 (28-84) |
| Smoking status, n (%) | Current smoker | 254 (46.7) |
| | Former smoker | 189 (34.7) |
| | Never-smoker | 101 (18.6) |
| Histology, n (%) | Adenocarcinoma | 255 (46.9) |
| | Squamous-cell carcinoma | 260 (47.8) |
| | NOS NSCLC | 29 (5.3) |
| *EGFR* mutation status, n (%) | Wild-type | 280 (51.5) |
| | Mutated | 34 (6.3) |
| | Unknown | 230 (42.3) |
| Line of treatment, n (%) | 1st line | 64 (11.8) |
| | 2nd line | 263 (48.3) |
| | 3rd and higher lines | 217 (39.9) |
| Natrium levels, n (%) | Low | 117 (21.5) |
| | Normal | 427 (78.5) |
| Stage, n (%) | IIIB | 114 (21.0) |
| | IV | 430 (79.0) |
| PS, n (%) | PS 0 | 8 (1.5) |
| | PS 1 | 296 (54.4) |
| | PS 2 | 216 (39.7) |
| | PS 3 | 24 (4.4) |

daily. The treatment was extended up until disease progression or development of intolerable toxic effects. In the event of treatment-related toxicity dose reduction or interruption was permitted.

*Follow-up monitoring*. The treatment was prospectively monitored and the clinical course of patients was continuously assessed at specific time points. Clinical follow-up controls including physical examination, plain chest X-ray and routine laboratory tests were performed every 3-4 weeks; computed tomography (CT) or positron emission tomography - (PET)-CT were performed after two or three months of treatment with erlotinib. The objective tumor response was assessed by investigators in terms of complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) using the Response Evaluation Criteria in Solid Tumours (RECIST). The disease control rate (DCR) was defined as the sum of CR, PR and SD. Progression-free survival (PFS) was determined from the date of erlotinib initiation up to the date of first documented progression or death. Overall survival (OS) was determined from the date of erlotinib initiation up to the date of death.

*Sodium levels measurement*. The concentration of sodium ions in serum was determined by indirect potentiometry (*i.e.* reconstituted serum) using a sodium ion selective electrode on the AU 5800 Beckman Coulter analyzer (Hostivar, Czech Republic).

*EGFR mutation analysis*. The tumor specimens acquired during initial bronschoscopy were evaluated by a senior cytologist using standard giemsa staining. In a few cases, a tumor biopsy was processed into formalin-fixed paraffin-embedded (FFPE) histological sections. The cytology slides or, eventually, the FFPE sections were submitted for molecular genetic testing, which included detection of somatic mutations in *EGFR* genes. If necessary, tumor cells were carefully selected and removed from the samples by laser microdissection using a P.A.L.M. microlaser instrument (Carl Zeiss MicroImaging GmbH, Jena, Germany). The microdissected cells were collected directly into the polymerase chain reaction (PCR) buffer and processed without a special DNA extraction step. In all other cases, the DNA was extracted from tissue cells by a standard spin-column procedure using the JetQuick Tissue DNA Issolation Kit (Genomed GmbH, Loehne, Germany). Mutations in exons 19 and 21 of the EGFR gene were tested by the Genoscan mutation detection kits (Genomac International, Prague, Czech Republic) utilizing a denaturing capillary electrophoresis (DCE) technique on an ABI PRISM 3100 16-capillary genetic analyzer (Applied Biosystems, Foster City, CA, USA). Detected mutations were confirmed by Sanger DNA sequencing using a BigDye v 3.0 chemistry (Applied Biosystems). In rare cases, where the overall fraction of mutated DNA was below the 20% threshold for DNA sequencing, the mutation was identified indirectly after forming only a homoduplex fragment with a given known mutation reference standard.

*Statistics*. Standard frequency tables and descriptive statistics were used to characterize a sample data set. The significance of differences between baseline characteristics, as well as treatment response and level of tumor markers, was determined using the Fisher's exact test. PFS and OS were calculated using the Kaplan Meier method and all point estimates were accompanied by 95% confidence intervals (CI). Statistical significance of the differences in the Kaplan-Meier estimates was assessed using the log-rank test. Univariate and multivariate Cox proportional hazards model was used to evaluate influence of all potential predictive and prognostic factors on the survival measures. As a level of statistical significance, $p=0.05$ was used.

## Results

*Sodium levels before treatment*. Before the begining of erlotinib treatment, low sodium level (Na ≤136 mmmol/l) was measured in 117 (21.5%) patients and normal sodium level was measured in 427 (78.5%) patients.

*Relation between natrium levels and treatment efficacy*. In patients with normal natrium level, CR was achieved in 5 (1.2%), PR in 45 (10.5%), SD in 209 (48.9%), PD in 152 (35.6%) patients and the objective tumor response was not evaluated in 16 (3.7%) patients. The DCR was 60.7%. In patients with low natrium levels, CR was achieved in 0 (0.0%), PR in 7 (6.0%), SD in 39 (33.3%) PD in 59 (50.4%) patients and the objective tumor response was not evaluated in 12 (10.3%) patients. The DCR was 39.3%. The difference between these two compared groups in DCR proved statistically significant ($p<0.001$). The median PFS for patients with normal natrium levels was 2.0 *vs.* 1.6 months for patients with low natrium levels ($p=0.001$). The median OS for patients with normal natrium levels was 10.9 *vs.* 4.6 months for patients with low natrium levels ($p<0.001$)

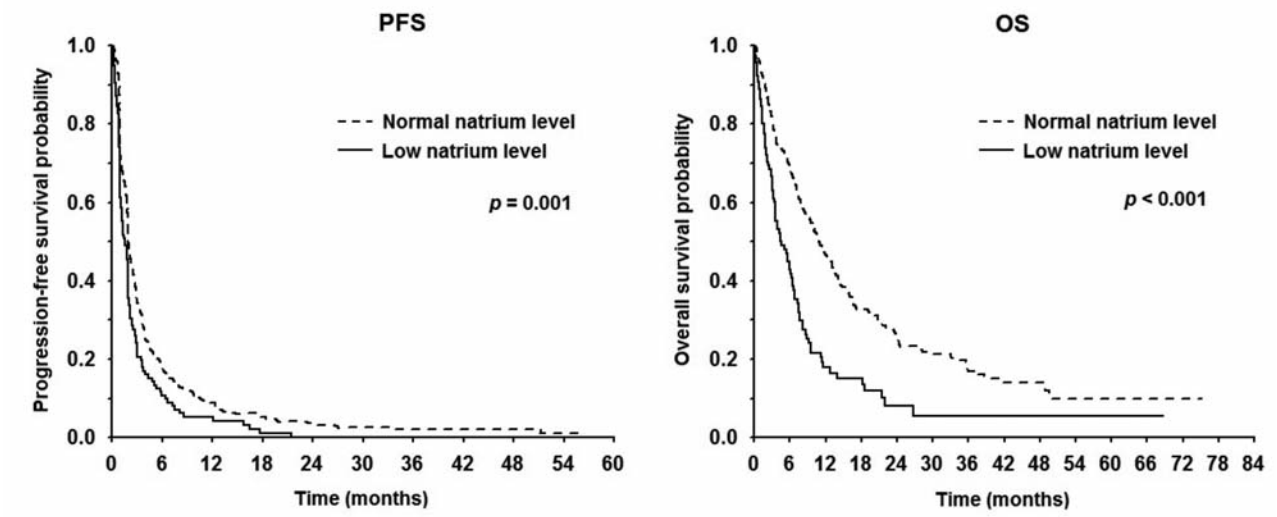


Figure 1. *Kaplan-Meier plots showing the comparison of progression-free survival (PFS) and overall survival (OS) according to natrium levels.*

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

| Parameter | Category | | PFS | | OS | |
|---|---|---|---|---|---|---|
| | | n | HR (95% CI) | *p*-Value | HR (95% CI) | *p*-Value |
| Gender | Males | 343 | 1.09 (0.90-1.33) | 0.389 | 1.25 (0.98-1.58) | 0.070 |
| | Females | 201 | 1.00 | | 1.00 | |
| Age | ≥65 years | 242 | 0.87 (0.73-1.04) | 0.137 | 0.94 (0.76-1.16) | 0.549 |
| | <65 years | 302 | 1.00 | | 1.00 | |
| Smoking | Current or former smoker | 443 | 1.21 (0.92-1.58) | 0.165 | 0.96 (0.7-1.32) | 0.814 |
| | Never-smoker | 101 | 1.00 | | 1.00 | |
| Histology | Adenocarcinoma | 255 | 1.20 (1.00-1.45) | 0.053 | 1.14 (0.91-1.43) | 0.247 |
| | Other | 289 | 1.00 | | 1.00 | |
| Stage | IV | 430 | 1.22 (0.98-1.53) | 0.073 | 1.49 (1.12-1.97) | 0.006 |
| | IIIB | 114 | 1.00 | | 1.00 | |
| ECOG PS | PS 2 or PS 3 | 240 | 1.28 (1.07-1.53) | 0.007 | 1.82 (1.47-2.25) | <0.001 |
| | PS 0 or PS 1 | 304 | 1.00 | | 1.00 | |
| Line | 3rd or higher | 217 | 0.96 (0.80-1.15) | 0.664 | 1.15 (0.92-1.42) | 0.215 |
| | 1st or 2nd | 327 | 1.00 | | 1.00 | |
| Natrium | Low | 117 | 1.25 (1.01-1.55) | 0.041 | 1.87 (1.47-2.39) | <0.001 |
| | Normal | 427 | 1.00 | | 1.00 | |
| EGFR | Mutated | 34 | 0.43 (0.29-0.66) | <0.001 | 0.58 (0.34-0.97) | 0.040 |
| Mutation | WT or unknown | 510 | 1.00 | | 1.00 | |

(Figure 1). The multivariate Cox proportional hazards model revealed that *EGFR* mutation status (HR=0.43, $p<0.001$), PS (HR=1.28, $p=0.007$) and natrium levels (HR=1.25, $p=0.041$) were significant independent factors for PFS, whereas the *EGFR* mutation status (HR=0.58, $p=0.040$), PS (HR=1.82, $p<0.001$), stage (HR=1.49, $p=0.006$) and natrium levels (HR=1.87, $p<0.001$) were significant independent factors for OS (Table II).

## Discussion

Hyponatremia has been reported in different carcinomas (8-10). Most of the published studies have focused on the topic of small cell lung cancer (SCLC), where hyponatremia is frequently caused by the syndrome of inappropriate antidiuretic hormone (SIADH) (10-13). The SIADH refers to a clinical syndrome caused by over-expressed secretion of

anti-diuretic hormone (ADH) (13) leading to high water re-absorption in kidneys. This proces leads to hyponatremia and serum hypo-osmolality (14).

The incidence of hyponatremia in patients with SCLC is about 20% for levels below 135 mmol/l and about 10% for levels below 130 mmol/l (12, 15, 16). On the other hand, only a few studies have focused on hyponatremia in NSCLC patients have been published.

In our study, we assesed hypontaremia in 21.5% patients with advanced-stage NSCLC. This is in agreement with Bose *et al*., who reported hyponatremia in 20% of NSCLC patients (17). On the contrary, several authors have reported lower incidence of hyponatremia in NSCLC patients (18, 19).

Low sodium plasmatic levels have been often mentioned as a negative prognostic factor in various malignancies. It is possible to find various studies about the influence of hyponatremia on prognosis of patients with gastric cancer, hepatocellular cancer, renall cell carcinoma and non-Hodgkin's lymphoma (9, 10). Mary studies have been published on the topic of SCLC. In a large mata-analysis by Castello *et al*. hyponatremia was significatntly associated with shorter survival duration in 7 of 13 studies (10). Only few published studies show hyponatremia as a negative prognostic factor in NSCLC patients (9, 17, 18, 20). However, Ray *et al*. did not confirm the prognostic significance of hyponatremia in NSCLC (21). It is important to mention that all these previously published studies enrolled different patient populations in comparison to our study. The studies published by Muers *et al*. and by Jacob *et al*. enrolled only untreated NSCLC patients (18, 20). The study by Kobayashi *et al*. enrolled patients undergoing surgical resection (9) and the last one by Bose *et al*., enrolled patients with SCLC and NSCLC together (17).

Until now, there has not been a published study focusing on the prognostic and predictive significance of low sodium levels (hyponatremia) in patients with advanced-stage NSCLC treated with EGFR-TKIs.

Our results showed significatntly shorter OS and PFS for patients with hyponatremia compared to those with normal sodium levels (4.6 *vs*. 10.9 months, $p<0.001$ and 1.6 *vs*. 2.0 months, $p=0.001$, respectively). Futhermore, we performed multivariate Cox proportional hazards model analysis to take into account other factors with possible influence on the prognosis and erlotinib treatment efficacy. This model confirmed that hyponatremia is an independent predictive factor for short PFS (HR=1.25, $p=0.041$) and also for short OS (HR=1.87, $p<0.001$).

The issue of predictive biomarkers has been a hot topic in oncology research. Activating *EGFR* mutations have been found as an important biomarker for prediction of high treatment efficacy of EGFR-tyrosine kinase inhibitors (TKIs) in NSCLC patients (22-25) and currently they are the only predictive biomarkers used in clinical practice. Therefore, patients harboring activating *EGFR* mutations are treated with EGFR-TKIs in the first-line (26). However, *EGFR* mutations are found only in 10-15% Caucasian patients (22, 23, 27). It is known that EGFR-TKIs are effective also at least in some patients harboring the wild-type *EGFR* gene who comprise the majority of patient population (28). Therefore, the search for new predictive biomarkers for EGFR-TKIs treatment is required. As mentioned above, we determined hyponatremia as not only a prognostic marker at NSCLC patients but also as a predictive marker of erlotinib treatment efficacy. It seems that hyponatremia predicts worse therapeutic response on EGFR-TKIs.

It is necessary to admit that there are several limitations of our study. The most important limitation is the retrospective design of our study. The next limitation is the cut-off value for hyponatremia. The cut-off value was different across previously published studies and, therefore, comparson of results is difficult. The most often cut-off value was defined as 136/135 mmol/l (9, 11-13, 17). Sodium levels of 136 mmol/l or less were determineted as hyponatremia also in our study.

In conclusion, we demonstrated hyponatremia as an independent factor for short PFS and also for short OS in patients with advanced-stage NSCLC treated with erlotinib in our large retrospective study. Its possible effect for use in clinical practice is bigger thanks to the simple possibility of testing hyponatremia and the low cost of this biomarker. More studies in this field are required to confirm our results.

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