Prognostic Value of Synaptophysin and Chromogranin A Expression in Patients Receiving Palliative Chemotherapy for Advanced Non-Small-Cell Lung Cancer

Dariusz M. Kowalski^a Maciej Krzakowski^a Piotr Jaśkiewicz^a
Włodzimierz Olszewski^b Anna Janowicz-Żebrowska^a Kamila Wojas-Krawczyk^c
Paweł Krawczyk^c

Departments of ^aLung Cancer and Chest Tumors and ^bPathology, Maria Skłodowska-Curie Memorial Cancer Center and Institute, Warsaw, and ^cDepartment of Pneumology, Oncology and Allergology, Medical University of Lublin, Lublin, Poland

Key Words

Non-small-cell lung cancer • Chemotherapy • Synaptophysin • Chromogranin A • Prognostic factors

Abstract

Background: Chemotherapy is the principal treatment method for patients with advanced non-small-cell lung cancer (NSCLC). Treatment with platinum-based and novel chemotherapeutic regimens, compared to monotherapy, slightly increases the response rates to 20–40%. The predictive and prognostic values of molecular factors are highly variable; however, data on clinical-demographic factors are still burdened by significant limitations. **Objectives:** The aim of this study was to assess the prognostic value of synaptophysin and chromogranin A protein expression in patients receiving palliative chemotherapy for advanced NSCLC. **Methods:** The study population consisted of 23 women and 116 men. The median age was 57.3 years. Expression of synaptophysin and chromogranin was assessed using a two-step model of immunohistochemical staining. Level 0 represented lack of activity, while level 1 represent-

ed its expression. **Results:** Expression of synaptophysin and chromogranin A was observed in 12 (8.6%) and 5 (3.6%) patients, respectively. The risk of death was significantly lower in patients with expression of synaptophysin ($p = 0.008$) and chromogranin A ($p = 0.014$). The 12- and 24-month survival rate of patients with synaptophysin expression was 64% (95% CI 0.35–0.93), while for patients without expression it was 46% (95% CI 0.36–0.56) and 16% (95% CI 0.07–0.25), respectively. The 12- and 24-month survival rate of patients with chromogranin expression was 80% (95% CI 0.44–1.00), while for chromogranin A-negative patients it was 47% (95% CI 0.37–0.57) and 19% (95% CI 0.10–0.28), respectively. We did not observe associations between expression of synaptophysin and chromogranin A and the other typical prognostic factors. **Conclusions:** Expression of synaptophysin and chromogranin A was associated with a longer median overall survival and might have prognostic value. These results should be confirmed in a prospective study.

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Introduction

Chemotherapy is the principal treatment method for patients with advanced-stage non-small-cell lung cancer (NSCLC). However, objective response rates range from 15 to 30% for monotherapy, whereas treatment with platinum-based or novel chemotherapeutic regimens slightly increases the response rates to 20–40% [1–4]. The benefits provided by chemotherapy, such as longer overall survival and improved quality of life, are predominantly observed in patients with good performance status [5]. In a study by Ginsberg et al. [6], the median overall survival was 9–11 months, while the 12- and 24-month survival rates were 20–40% and 5–10%, respectively. These results are associated only with cisplatin-based chemotherapy regardless of the regimen type. Chemotherapy also constitutes a valuable therapeutic approach in palliative patients above 70 years of age with good performance status without concomitant severe diseases [7, 8], but its efficacy in patients with poor performance status remains controversial [9–11]. Current recommendations of the Polish Group of Lung Cancer do not recommend treatment longer than 4 cycles of chemotherapy in patients with an objective response [12–14].

Negative prognostic factors in patients with advanced stages of NSCLC are: poor performance status assessed according to Karnofsky's or Zubrod's scale, significant weight loss, advanced age, worse clinical staging (T trait from TNM classification), involvement of regional lymph nodes (N from TNM classification), presence of metastases (M parameter from TNM classification), and elevated activity of lactic acid dehydrogenase (LDH) and decreased hemoglobin (Hb) concentration detected prior to treatment. The prognostic value of other factors such as: leukocyte count, platelet count, calcium serum concentration, gender, and the presence of paraneoplastic syndrome has not been confirmed yet [15, 16]. However, some of them (poor performance status or advanced age) might affect the outcome of chemotherapy. The predictive value of the histologic type of cancer for the efficacy of different drugs or chemotherapy regimens has been already proven [15]. Summarizing, the predictive and prognostic values of mentioned factors are highly variable, especially when the clinical-demographic factor data is still burdened by significant limitations. What is more, additional specific molecular or genetic factors must be found. The aim of this study was to assess the prognostic value of synaptophysin and chromogranin A protein expression in patients receiving palliative chemotherapy for advanced NSCLC.

Material and Methods

The prognostic value of synaptophysin and chromogranin A expression was retrospectively analyzed in 139 palliative patients with locally advanced and advanced stage NSCLC. Study population consisted of 23 women (16.5%) and 116 men (83%). The median age was 57.3 years (range 31–75). The inclusion criteria for our study were: availability of tumor tissue sufficient for immunohistochemical analysis, and accessible clinical data throughout observation during and after first-line chemotherapy. Measurements of the expression of both proteins and histologic reevaluation of cancer features were performed in the Pathology Department of the Maria Skłodowska-Curie Memorial Cancer Center and the Institute of Oncology in Warsaw, Poland. Clinical data were collected from documentation of palliatively treated patients with stage III or IV NSCLC. Patients who had previously received radical radio- and radiochemotherapy were disqualified. The clinical characteristics of patients are shown in table 1. Clinical staging was performed according to the TNM classification with use of physical examination, chest X-ray, bronchofiberscopy, computed tomography (CT) of the chest, abdominal ultrasonography, and, in several cases, scintigraphy of the skeletal system and MRI or CT of the central nervous system. Performance status according to Karnofsky's scale, weight loss, LDH activity, and Hb concentration in the peripheral blood were entered into the analysis of classical prognostic factors.

All patients were treated with platinum-based chemotherapy. However, 59 patients (42.2%) received a regimen of cisplatin and etoposide and 32 patients (23%) were treated with a regimen of cisplatin and gemcitabine, whereas in 8 patients (5.8%) a regimen of cisplatin and vinorelbine was used. In other cases, the regimens consisted of cisplatin with vinorelbine, and carboplatin with vinorelbine; gemcitabine, etoposide, and paclitaxel were used less frequently. The number of therapeutic cycles ranged from 1 to 11 (1 cycle, 2 patients, 1.4%; 11 cycles, 1 patient, 0.7%) with a mean number of 4 cycles. Response to treatment was assessed according to WHO criteria after administration of 2, 4, and the last cycle. Second-line treatment was administered in patients with progressive disease (PD) or symptoms associated with cancer. The majority of them (37.4%, $n = 52$) received radiotherapy, while 14 patients (10.1%) were treated with second-line chemotherapy. The remainder received best supportive care. At the time of the analysis, 87 patients (62.6%) had died and 5 were still alive. Because in 47 patients no survival data were found, to determine the treatment results the date of the latest control examination was used in the analysis.

Expression of neuroendocrine markers (synaptophysin and chromogranin A) was assessed using a two-step model of immunohistochemical staining. Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from all examined patients. Laboratory measurement of markers was performed using DAKO Antibody Assays anti-chromogranin A (M0869) and anti-synaptophysin (M0776). Immunohistochemistry staining was performed at room temperature using a humidity chamber and then incubation with a high-sensitivity detection kit according to the manufacturer's instructions. The sections were dewaxed with xylene, rinsed in graded ethanol, and rehydrated in water before blocking of endogenous peroxidase activity with 3% H_2O_2 for 10 min. Antigen retrieval was achieved by heating the slices in 0.01 M citrate buffer, pH 6.0, at room temperature. An

Table 1. Baseline characteristics of the patients

	Patients n	% actual	% population
Gender			
Female	23	16.5	16.5
Male	116	83.5	83.5
Performance status			
80–100	120	86.3	86.3
<80	19	13.7	13.7
T parameter			
TX	5	3.6	3.6
T1	3	2.2	2.2
T2	31	22.3	22.3
T3	50	36.0	36.0
T4	50	36.0	36.0
N parameter			
NX	10	7.2	7.2
N0	8	5.8	5.8
N1	21	15.1	15.1
N2	56	40.3	40.3
N3	44	31.7	31.7
M parameter			
M0	91	65.5	65.5
M1	48	34.5	34.5
Clinical staging			
IIIA	28	20.9	20.9
IIIB	63	45.3	45.3
IV	48	33.8	33.8
Localization of metastases			
Liver	6	12.5	4.3
Skeletal system	4	8.3	2.9
Central nervous system	5	10.4	3.6
Adrenal gland	7	14.6	5.0
Lymph nodes	11	22.9	7.9
Liver + adrenal gland	12	25.0	8.6
Other	3	6.3	2.1
Number of metastases			
1	16	33.3	11.5
>1	32	66.7	23.0
Histologic type			
Squamous-cell carcinoma	92	66.2	66.2
Adenocarcinoma	26	18.7	18.7
Large cell carcinoma	3	2.2	2.2
Undetermined type of NSCLC	18	12.9	12.9
Grading			
G1	9	6.5	6.5
G2	105	75.5	75.5
G3	25	18.0	18.0

FFPE section of human carcinoid with antibody dilution from 1:10 to 1:20 served as a positive control for synaptophysin staining, while an FFPE section of human colon with antibody dilution from 1:100 to 1:200 served as a positive control for chromogranin A. The mouse IgG1 monoclonal antibody and mouse IgG2b monoclonal antibody against *Aspergillus niger* glucose

Table 2. Expression of synaptophysin and chromogranin A in the study population

Level of expression	Patients, n	Patients, %
Synaptophysin		
0	127	91.4
1	12	8.6
Chromogranin A		
0	134	96.4
1	5	3.6

oxidase served as a negative control for synaptophysin and chromogranin A staining, respectively. Level 0 represented lack of activity, while level 1 represented expression of these proteins in cancer cells [17].

The probability of survival was assessed using the Kaplan-Meier method. Identification of prognostic factors was performed using a Cox proportional hazards model. In the first step, the model was adjusted with classical prognostic factors such as: gender, T parameter, N parameter, M parameter, staging according to the TNM classification, weight loss, performance status according to Karnofsky's scale, histological type, grading, LDH activity, and Hb concentration. Backward stepwise variable selection was used for model building. Single factor assessment was performed using cross-tabulation and χ^2 .

The study was approved by the Ethical Committee of the Medical University of Lublin (decision No. KE-0254/142/2010).

Results

Expression of synaptophysin and chromogranin A was observed in 12 (8.6%) and 5 (3.6%) patients, respectively, whereas lack of their activity was noted in 127 (91.4%) and 134 (96.4%) patients, respectively. All chromogranin A-positive tumors were simultaneously positive for synaptophysin staining. These results are shown in table 2.

To determine the association between histologic type and expression of synaptophysin and chromogranin A, the study population was divided into two subgroups: those with squamous cell carcinoma and those with adenocarcinoma. A group of 21 patients with large cell carcinoma or an undetermined histologic type of tumor was excluded from the analysis which was finally performed in a group of 118 patients. No significant relation between histologic type and expression of neuroendocrine markers was confirmed in the study group ($p = 0.083$ for synaptophysin and $p = 0.322$ for chromogranin A, respectively). Seven patients with squamous cell carcinoma and

Fig. 1. Overall survival of the study population.

Fig. 2. Overall survival depending on expression of synaptophysin.

5 patients with adenocarcinoma expressed synaptophysin. Expression of chromogranin A was detected in 3 patients with squamous cell carcinoma and in 2 patients with adenocarcinoma. Moreover, no significant relation between gender and expression of the analyzed markers was found ($p = 0.991$ for synaptophysin and $p = 0.311$ for chromogranin A, respectively).

To determine the relation between staging of disease according to the TNM scale and expression of synaptophysin and chromogranin A, the study population was divided into two subgroups: those with T1 or T2 stage and those with more advanced stages of disease including T3 or T4. No significant relation between T parameter characteristics and expression of synaptophysin ($p = 0.468$) and chromogranin A ($p = 0.778$) was revealed. The association of synaptophysin or chromogranin A expression with lymph node involvement and distant metastasis characteristics was assessed in 129 and 139 patients, respectively. No association was noted in either group (the p value for the relation of synaptophysin and chromogranin A expression in the first group was 0.694 and 0.46, while in the second group the p value was 0.927 and 0.486, respectively). The relation between clinical staging of the disease and marker expression was determined in all 139 patients. However, no significant link was noted ($p = 0.916$ for synaptophysin and $p = 0.763$ for chromogranin A).

In most of patients (56.1%, $n = 78$) the outcome was stable disease (SD) while complete response (CR), partial response (PR), and PD were documented in 4 (2.9%), 44 (31.75%), and 13 (9.4%) patients, respectively. Synaptophysin was expressed in 1 patient with CR, in 3 patients with PR, in 7 patients with SD, and in 1 patient with PD (insignificant). Moreover, 3 patients with SD, 1 patient with PR, and 1 patient with PD expressed chromogranin A (insignificant).

The median of overall survival in the study population was 11.7 months (range 1.01–43.47). The curve of overall survival is illustrated in figure 1. The probability of 12- and 24-month survival was: 0.48 (95% CI 0.38–0.58) and 0.22 (95% CI 0.13–0.31), respectively. The relation between overall survival and synaptophysin expression was analyzed in two subgroups: with and without marker expression. The risk of death was significantly lower in patients with expression of synaptophysin ($p = 0.008$). The 12- and 24-month survival in these patients was 64% (95% CI 0.35–0.93) and 64% (95% CI 0.35–0.93), respectively. In patients without expression of synaptophysin, the 12- and 24-month survival was 46% (95% CI 0.36–0.56) and 16% (95% CI 0.07–0.25). These results are shown in table 3 and figure 2.

The relation between overall survival and chromogranin A expression was analyzed in two subgroups: with and without marker expression. The risk of death was

significantly lower in patients with presence of chromogranin A expression ($p = 0.014$). The 12- and 24-month survival in these patients equaled 80% (95% CI 0.44–1.00) and 80% (95% CI 0.44–1.00), respectively. In patients negative for chromogranin A, the 12- and 24-month survival was 47% (95% CI 0.37–0.57) and 19% (95% CI 0.10–0.28), respectively. These results are shown in table 4 and figure 3.

Discussion

Chromogranin A is a glycoprotein with a molecular mass of 48 kDa located in secretory high-density vesicles of neuroendocrine cells also named APUD (amine precursor uptake and decarboxylation cells). Potentially biologically active peptides derived from chromogranin A are: vasostatin, chromostatin, chromacins, pancreastatin, WE-14, catestatin, parastatin, and GE-25. Although chromogranin A is detected in normal tissues as well as in many type of cancers, SCLC and neuroendocrine tumors show particularly strong expression of this protein [18, 19]. In most patients with carcinoids (56–100%) increased levels of chromogranin A are observed; however, the expression profile depends on the mass of the tumor and might be considered as a prognostic factor [20, 21]. Assessment of chromogranin A expression is also a routinely performed diagnostic procedure in: SCLC, medullary thyroid cancer, neuroblastoma, Merkel cell carcinoma, and primitive neuroectodermal tumors [22]. Synaptophysin is a membrane glycoprotein with a molecular mass of 38 kDa encoded by the gene located on chromosome X (p11.23p–11.22) [16].

Neuroendocrine features of tumor defined as expression of synaptophysin and/or chromogranin A are found only in 10–30% of patients with NSCLC. Evaluation of chromogranin A and synaptophysin expression is routinely performed in the diagnostic procedure in case of SCLC or carcinoids, but its applicability in diagnosis of NSCLC remains unclear. The influence of neuroendocrine features of NSCLC on the prognosis and probability of response to treatment is difficult to assess. It results from the fact that a small group of patients with NSCLC (0–20%) shows positive expression of chromogranin A, synaptophysin, or neuron-specific enolase in comparison with patients with SCLC [23]. However, neuroendocrine features of NSCLC are considered risk factors for more aggressive course of disease (characterized by: a more advanced clinical stage, a higher incidence of metastases, and resistance to chemotherapy) [24–27].

Fig. 3. Overall survival depending on expression of chromogranin A.

Table 3. Coefficients of the Cox model with the parameter of synaptophysin expression

	B	Standard error	p (Wald)	Exp(B) relative risk
Performance status	0.494	0.174	0.005	1.639
Weight loss >10%	0.628	0.254	0.014	1.172
Histologic type			0.020	
Squamous cell carcinoma	1.005	0.360	0.005	2.731
Adenocarcinoma	0.737	0.421	0.080	2.091
LDH above the normal level	0.526	0.273	0.054	1.692
Synaptophysin	–1.522	0.573	0.008	0.218

Table 4. Coefficients of the multivariate Cox model including chromogranin A expression

	B	Standard error	p (Wald)	Exp(B) relative risk
Performance status	0.595	0.177	0.001	1.813
Weight loss >10%	0.648	0.255	0.011	1.912
Histologic type			0.013	
Squamous cell carcinoma	1.048	0.364	0.004	2.853
Adenocarcinoma	0.619	0.420	0.140	1.858
LDH above the normal level	0.554	0.272	0.042	1.741
Chromogranin A	–1.960	0.800	0.014	0.141

The prognostic and predictive value of neuroendocrine features of NSCLC has been determined in a number of multicenter studies. Graziano et al. [28] assessed the expression of chromogranin A and synaptophysin in 160 patients with inoperable NSCLC. None of them presented expression of chromogranin A, while expression of synaptophysin was confirmed in 15% of cases. Petrovic et al. [29] found expression of neuron-specific enolase in 22.4%, chromogranin A in 15.5%, and synaptophysin in 14.8% of tumor cells from locally advanced and advanced NSCLC patients. In their study, the response to chemotherapy was significantly better in patients with NSCLC with neuroendocrine differentiation. Moreover, the percentage of tumor cells with the ability to produce neuroendocrine markers was a significant independent prognostic factor associated with favorable outcomes.

Similar results indicating a low level of neuroendocrine marker expression were found in our study (synaptophysin expression was found in 4.7% of women and 8.6% men, respectively; chromogranin A was found only in 4.3% of men). However, very small chromogranin A- and synaptophysin-positive arms (12 and 5 patients, respectively) in our study limited their prognostic and predictive value in chemotherapy-treated patients. In our study population, a five-fold reduction in risk of death was observed for synaptophysin and the reduction was seven-fold for chromogranin A. Comparable results have been reported by other authors [30–34]. It is also important to indicate that our study was limited due to the absence of survival data for 47 (33%) patients. Nonetheless, most differences in comparison with our study resulted from the disease stage profile: most of the quoted studies analyzed neuroendocrine marker expression in patients with stage III/IV disease that underwent chemotherapy, while our analysis covered only palliative patients. A better prognosis in patients with neuroendocrine features of tumor was not associated with higher response rates. However, use of a broader spectrum of neuroendocrine parameters could potentially increase the sensitivity to chemotherapy [28].

As in other studies, we did not find any significant association between the histologic type of NSCLC and neuroendocrine marker expression [35–39]. However, expression of synaptophysin was insignificantly more frequently observed in patients with adenocarcinoma than in patients with squamous cell carcinoma. In other studies, elevated expression of neuroendocrine features of lung adenocarcinoma has been noted in patients who were subjected to radical surgical treatment [25, 28, 30, 40]. Similarly to the results obtained by Yu et al. [41], we did not observe any relation between marker expression and regional lymph node involvement. Although Sundaresan et al. [42] showed a significant correlation between endocrine features of tumor and clinical staging, including lymph node involvement, it did not translate into a similar association between overexpression of neuroendocrine features and overall survival. However, most of the data found in previous studies was derived from surgically treated patients [28, 43–46]. None of the currently published articles about advanced and inoperable NSCLC have shown relations between neuroendocrine characteristics and T parameters depending on clinical staging or gender. In our study group, no significant relation with these factors was detected.

Conclusions

In our study population, we did not observe significant associations between expression of synaptophysin and chromogranin A and other classical prognostic factors. However, expression of synaptophysin and chromogranin A was associated with longer median overall survival and might have a prognostic value. These results should be confirmed in a prospective study.

Financial Disclosure and Conflicts of Interest

The authors report no conflict of interest.

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