CHROMOGRAFIN A TISSUE EXPRESSION AS A PROGNOSTIC FACTOR IN ADVANCED NON SMALL CELL LUNG CANCER

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ABSTRACT

To determine the frequency of chromogranin A (CgA) and influence on survival of treated patients with advanced non small cell lung cancer (NSCLC). This study included 236 patients with histological diagnosis of advanced NSCLC (III and IV disease stage). Combined chemotherapy and radiotherapy protocol was used in III stage of disease (without pleural effusion) where as chemotherapy was used in III stage (with pleural effusion) as well as in IV stage of disease. Immunohistochemical analysis of CgA tissue expression was determined in tissue assays using antibodies to CgA. The overall survival of patients was assessed in one year and two years follow-up period. Of 236 eligible patients, 36 (15,25%) had CgA expression. Squamous cell lung carcinomas had the least frequency of CgA tissue expression (8,7%). The 1-year and 2-year survival rates were 64% and 27% in group of patients with CgA expression compared to 32% and 6% in group without CgA expression (log-rank test:p<0.001). The median survival time in group of patients with and without positive CgA expression was 15.7 vs 12.3 months, respectively. One year survival rate was higher in NSCLC patients with more than 50% of CgA positive cancer cells (log-rank test: p<0.001).

Key words: non small cell lung cancer, neuroendocrine expression, chromogranin A, frequency, survival

SAŽETAK:

Ispitivana je učestalost homogranina A (CgA) i njegov uticaj na preživljavanje kod lečenih bolesnika sa odmaklim nesitnočelijskim karcinomom pluća. U studiju je uključeno 236 bolesnika sa histoloskim dijagnostom NSCLC (III i IV stadijum bolesti). Kombinovana hemio i radiotherapija bila je uključena u III stadijumu bolesti (bez pleuralnog izliva), a samo hemoterapija u III (sa pleuralnim izlivom) i IV stadijumu bolesti. Za imunohistohemijsku analizu tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA. Preživljavanje pacijenata praćeno je u jednogodišnjem i dvogodišnjem periodu. Od ukupna 236 ispitivanih pacijenata, 36 (15,25%) imalo je ekspresiju CgA. Najmanju učestalost tkivne ekspresije homogranina A korišćena su miša, monoklonalna antitela na CgA.

1. INTRODUCTION

Lung cancer is the leading cause of cancer death in the world. Non-small cell lung carcinoma (NSCLC) accounts for about 80% of all lung cancers. A high level of chemotherapy and radiotherapy resistance is described in non small cell lung cancer but 5-year overall survival rate was only 14% (1). Differing survival outcomes among patients within a stage suggests the existence of other tumor factors affecting prognosis (2). In the past two decades there have been substantial changes in concepts regarding the nature of lung tumors showing neuroendocrine (NE) differentiation (3). Immunohistochemistry (IHC) is the most practical method of assessing protein expression changes in histopathology. IHC not only provides a semiquantitative assessment of protein abundance but also defines the cellular localisation of expression. These considerations have led to the extensive use of IHC in studies on prognostic markers for tumors (2).

IHC studies indicated that NE features are expressed by 10-30% of ordinary NSCLC (4, 5, 6) especially adenocarcinomas, large cell carcinomas and squamous cell carcinomas, all traditionally considered of non-
The study included 236 patients with histological stage III-IV NSCLC with NE differentiation (NSCLC-NE). They are characterized by panendocrine expression, neuroaminergic, and neuropeptids and have ultrastructural pattern of specific secretory granules confirmed using immunohistochemical method or by electronic microscopy (7). Clinical and therapeutic significance NSCLC-NE has not been firmly established (3).

Recent studies in which neuroendocrine expression was described as prognostic factor, have provided inconsistent and sometimes conflicting results. Some series have shown NSCLC-NE to be associated with longer survival (5,8), whereas others have not (9,10). Carnaghi et al. confirmed these controversial data, analyzing 13 large clinical trials. In two of them, authors described shorter survival, in eight studies expression did not correlate with survival, but in rest three there were significantly longer survivals (11).

Chromogranins are the major proteins in peptide containing dense core (neurosecretory) granules, and antibodies against these are the most specific markers of NE differentiation (6). Chromogranin A (CgA) is a high molecular weight acidic glycoprotein originally isolated from adrenal medulla. It is released along with neuroendocrine peptides through exocytosis from dense-core neurosecretory granules and its detection is directly correlated with the presence of these neurosecretory granules (12). It has been found that a broad spectrum of immunohistochemical markers can highlight neuroendocrine (NE) differentiation in lung tumors, although CgA remain the most strikingly consistent general marker due to its close correlation with the ultrastructural evidence of neurosecretory granules and small clear vesicles, respectively.

The goal of the current study was to determine frequency and influence of CgA expression on survival of treated patients with advanced NSCLC.

2. PATIENTS AND METHODS

2.1. Patients

The study included 236 patients with histological stage III-IV NSCLC, diagnosed and treated at Military Medical Academy, Belgrade and Clinical Center Kragujevac between January 2001 and December 2006. The disease was classified according to the revised International System for Staging Lung Cancer (13). Staging was performed prior to the most recent update of the therapy protocol. Therapy was determined according to disease stage. Patients with IIIA and IIIB stage of disease (without pleural effusion) were treated with combined chemotherapy and radiotherapy. Combined cisplatin-carboplatin chemotherapeutical protocol (not more than six cycles) was conducted until the disease progression (increase more than 20% in measurable tumor). When the progression was noted, the treatment was continued with radiotherapy only (Split course, TD 55-60Gy). Patients with IIIB (with pleural effusion) as well as with IV stage of disease were treated only with chemotherapy. Survival of treated patients was assessed at 1-year and 2-year follow up period.

2.2. Histology and immunohistochemistry

Formalin-fixed and wax-embedded tumor tissues was cut into 4 μm-thick sections and mounted on slides (Super Frost® Plus, Braunschweig, Germany). Regular hematoxylin and eosin (H&E) staining was used for classification according to the WHO classification system for lung carcinoma (14).

Sections for IHC were dewaxed with xylene, rinsed in graded alcohol, rehydrated in water, and immersed in 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Antigen retrieval was achieved by heating the sections in a microwave (Panasonic NN-252W) for 20 min in 0.5M citrate buffer (pH 6.0). The sections were incubated with the anti-human CgA (1:100, M0869, DakoCytomation, Glostrup, Denmark) for 20 h at 4 °C. Between each step the sections were washed in TBS with 0.05% Tween 20, and the immunoreactivity was visualized using Envision® (K5007, DakoCytomation, Glostrup, Denmark) . A pancreatic tissue was used as positive control.

Assessments of staining intensity (0 = none, 1+ = weak, 2+ = moderate, 3+ = strong) and percentage of tumor cells positive (0 = none, 1+ = <10%, 2+ = 10-50%, 3+ = >50%) were made (15). For CgA antibody the score for intensity was multiplied by that for distribution to give an intensity-distribution (ID) score. An ID score of >2 was used as the criterion for evidence of CgA tissue expression.

2.3. Statistics

Patients’ overall survival time was defined as the interval from date of diagnosis to death or to last contact for living patients. Overall survival was graphically presented using Kaplan–Meier method. The log-rank test was used to analyse patients’ survival data between groups. Median overall survival time and the 1-year survival rate were obtained from the Kaplan–Meier curves. The Chi-square (χ²) test was used to compare differences in patients’ characteristics between groups. The level of statistical significance was defined as p<0.05.

3. RESULTS

3.1. Patient’s characteristics

A total of 236 patients with advanced non small cell lung cancer were examined. The patient’s characteristics were shown in table 1. The median age of the patients was 62.35±11.57 years (SD), range 37-74. The majority of them (42.41%) belonged to 60-69 years cohort group. One hundred and seventy four (73.72%) of the patients
Of 115 patients with squamous cell carcinomas only 10 (8.7%) had CgA expression. The frequencies of CgA expression in group of patients with adenocarcinomas, large cell carcinomas and adenosquamous carcinomas were 20.78%, 33.3% and 17.24%, respectively. There was a positive correlation between CgA expression and NSCLC histological types (p<0.001).

3.3. Survival rate analysis

The 1-year survival rate was 64% in group of patients with CgA tissue expression compared to 32% in group without CgA tissue expression (Graph 1). The 2-year survival rate was 27% in group of patients with CgA tissue expression compared to 6% in group without CgA tissue expression (Graph 2). There was a positive correlation between survival rate of treated patient and CgA tissue expression in the 1-year (log-rank test: p< 0.001) and 2-year follow-up period (log-rank test: p< 0.001). Patients with a CgA expression had a median overall survival time longer than those without this neuroendocrine marker expression, 15.7 versus 12.3 months, respectively.

Histogramically, one hundred and fifteen (48.72%) were classified as squamous cell carcinoma, 77 (32.63%) were adenocarcinomas, 15 (6.36%) were large cell carcinomas and 29 (12.29%) were adenosquamous carcinomas.

3.2. Chromogranin A characteristics

Of 236 eligible patients, 36 (15.25%) had CgA expression. Distribution of CgA expression in relation to NSCLC histological types is demonstrated in Table 2.

Table 1. Patient’s characteristics

Table 2. CgA expression distribution in relation to NSCLC histological type

Graph 1. The 1-year survival rate curve of NSCLC patients with CgA positive and negative tissue expression
Analysis of one-year and two-year median survival time in patients group A (III A + III B stage, without pleural effusion) and group B (III B with pleural effusion and IV stage) showed statistically significant difference in survival (p = 0.000). One-year (62%) and two-year (30%) median survival time was longer in patients group A compared to group B, where 38% of patients lived one year and more, but only 5% of treated patients lived for 24 months (Graph 5).

4. DISCUSSION

The aim of the present investigation was to analyse frequency of chromogranin A tissue expression and impact of this strikingly consistent neuroendocrine marker on survival of treated patients with advanced non small cell lung cancer.

For this purpose, expression of CgA, which is strongly associated with neuroendocrine differentiation in NSCLC, have influence on median survival time in patients with NSCLC of stage III and IV are stage of disease (p<0.001) and percentage of positive tumor cells (p=0.000).

The 1-year survival rate of patients with more than 50% of CgA positive cancer cells was 100%. Survival time between patients with less than 10% of CgA positive cancer cells was no longer than 5 months (Graph 3). There was significant difference in the 1-year (Graph 3) and 2-year (Graph 4) survival time and percentage of CgA positive cancer cells (log-rank test; p<0.001).

Multivariate binary logistic regression was used to assess simultaneous influence of all parameters to median survival time. (Table 3). Parameters which are proved to have influence on survival time in patients with NSCLC

### Table 3. Influence of parameters on survival time in patients with NSCLC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influence on Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of Disease</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Percentage of CgA Positive Cancer Cells</td>
<td>p=0.000</td>
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</tbody>
</table>

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nomas (2/9), and one of the large cell carcinomas (1/2) (18). A considerable overlap occurred in all histological groups, a finding which contrasts with those of other workers who found that NE markers were more commonly expressed in adenocarcinomas and only rarely in squamous cell carcinomas (23).

In the present study, we investigate the impact of CgA expression on prognosis, indicating that NE differentiation is a significant prognostic factor.

In the survival analysis, we found that the presence of CgA tissue expression significantly correlated with survival. The 1-year and 2-year survival rate were significantly higher in group of patients with CgA expression compared to those without expression of this neuroendocrine marker. Median survival time was higher in group of patients with CgA tissue expression compared to those without expression.

Even though some studies have shown a prognostic significance of NE differentiation in subgroups such as adenocarcinomas (17, 22), the present opinion is that the finding of some tumor cells with NE features does not seem to influence prognosis or response to treatment (6, 15, 24).

In a review of the literature, Schleusener et al. (5) reported that NE differentiation in NSCLC has been shown in different studies to be associated with either: improved survival (mostly in chemotherapy-treated patients) or decreased survival (mostly in surgically treated patients), or to have no bearing at all on survival.

Using multivariate analysis, Abbona et al. (23) showed that NE differentiation in NSCLC had a negative impact on survival and was predictive of higher disease stage.

Carnaghi et al. reviewed 13 major studies investigating either the prognostic or predictive value of neuroendocrine differentiation in NSCLC. This review showed that there are conflicting results regarding the importance of neuroendocrine differentiation; two studies showed decreased survival, eight showed no correlation with survival, and three showed improved survival (11).

Skov et al. analyzed percentage of CgA positive cancer cells as a prognostic factor in NSCLC patients. Patients with more than 10% of CgA positive cancer cells have a longer survival time compared to those with less percentage of positive cells (12). In our study, one year survival rate in group of NSCLC patients with more than 50% CgA positive cancer cells was longer compared to group with less than 10% of CgA positive cancer cells.

Further work is needed to assess their usefulness as NE markers and to see whether they might provide additional information on NE differentiation and prognosis.

Obviously, lung cancer remains a frustrating clinical problem, notorious for poor treatment results.
5. Conclusion
Chromogranin A tissue expression was registered in 15.25% of patients with advanced non small cell lung cancer with neuroendocrine differentiation. Large cell lung cancers had the highest frequency of CgA tissue expression compared to other histological cancer types. The 1-year and 2-year survival time were longer in patients with CgA expression. Moreover, longer survival time was often associated with presence of more than 50% of chromogranin A positive cancer cells. Therefore, CgA tissue expression was associated with improved patient’s survival.

REFERENCES