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Immunohistochemical expression of HER-2/neu in patients with lung carcinoma and its prognostic significance

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Background. *The HER-2 protein or p185her2 is a membrane receptor with tyrosine kinase activity encoded by HER-2/neu gene. Overexpression of HER-2/neu has been observed in many human cancers, including lung cancer. In the study, the expression of HER-2 protein is determined in the spectrum of lung cancer (adenocarcinoma, squamous cell carcinoma and small cell carcinoma).*

Patients and methods. *The study population consisted of two groups: 19 patients that had undergone surgical treatment and 10 patients that had undergone fiber-optic bronchoscopy and biopsy for primary diagnosis only. Tissue specimens were neutral formaldehyde-fixed and paraffin-embedded. Standard histochemical and immunohistochemical staining were used for diagnosis. Expression of HER-2/neu protein was determined by immunohistochemical staining with Hercep Test™ (DAKO). The results were graded 0-1 as negative and 2-3 as positive.*

Results. *Overall incidence of HER-2/neu overexpression was 34.4% (10 of 29). Higher incidence was found in the patients with adenocarcinoma 45.4% (5 of 11). In squamous cell carcinoma and small cell carcinoma, the overexpression incidence was 30.7% (4 of 13) and 20% (1 of 5), respectively. No statistically significant difference was seen given the age and gender. HER-2/neu overexpression was more pronounced in the patients with advanced tumour: all patients with squamous cell carcinoma and HER-2/neu overexpression had stage IIIB and stage IV disease, while 80 % of adenocarcinoma patients with HER-2/neu overexpression had stage IIIA and IIIB disease.*

Conclusions. *These results are satisfactory and encourage us to continue this work in the follow-up study to evaluate HER-2/neu role as predictive and prognostic factor for the patients with lung cancer.*

Key words: lung neoplasms – pathology; receptors,, erb-2; prognosis; immunohistochemistry

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Introduction

Lung cancer is the leading cause of cancer mortality worldwide. In Macedonia, lung cancer is the cause of death in nearly 19.44% of all cancer deaths.[1] Most of the patients had

an advanced stage of the disease at the time of diagnosis. Even with early diagnosis and an adequate treatment, the 5-year survival rate of the patients with stage I disease is about 70%.[2]

We presume that there is an intrinsic factor that determines the clinical course of the disease. Advances in molecular pathology showed that expression of p53, c-myc, c-fos, c-erbB-1,[3] and Her-2/neu[4,5] is associated with lung cancer. The HER-2 protein or p185-her 2 is a membrane receptor with tyrosine kinase activity encoded by HER-2/neu gene.

HER-2/neu overexpression has been observed in many human cancers, including carcinoma of the breast, ovary, gastrointestinal tract, salivary gland and lung.[6] There are different reports about the expression of this protein in different lung cancers.

The aim of the study was to determine the expression of HER-2 protein in the adenocarcinoma, squamous cell carcinoma (squamous CC) and small cell carcinoma (small CC) of the lung, and to assess the correlation between HER-2/neu expression and clinical stage of the patients.

The evaluation of the expression of HER-2/neu is important because of its prognostic significance and possibility to treat the patients with trastuzumab (Herceptin™).

Material and methods

The study population consisted of two groups: 19 patients that had undergone surgical treatment and 10 patients that had undergone fiber-optic bronchoscopy and biopsy for primary diagnosis only.

The stage of the disease was classified according to the new international staging system for lung cancer, using the following clinical investigations: blood examination, biochemical studies, chest radiography, whole bone scan and computed topographic scan of the chest.

Tissue specimens from surgical and biopsy material were neutral formaldehyde-fixed and paraffin-embedded. Standard histological stainings (Hemalaun-Eosin, PAS, Alcian blue –PAS and reticulin – Gomori) were used for diagnosis.

HER-2/neu oncogene expression was determined by immunohistochemical staining with Hercep Test™ (DAKO). The slides were deparaffinized in xylene and rehydrated with graded ethanol. Following the incubation with the primary rabbit antibody to human HER-2 protein, ready-to-use Visualisation Reagent consisted of both secondary goat anti-rabbit immunoglobulin and horseradish peroxidase, was used. The enzymatic conver-

Table 1. Distribution of the patients according to the histological type of the tumour and clinical stage

Histological type	Clinical stage					Total
	I	II	IIIa	IIIb	IV	
Adenocarcinoma	0	5	3	3	0	11
Squamous CC	0	5	2	4	2	13
Small CC	0	2	3	0	0	5
Total	0	12	8	7	2	29

Table 2. Overexpression of HER2 according to histological type and clinical stage of disease

Histological type HER2+/total	Clinical stage					Total (%)
	I	II	IIIa	IIIb	IV	
Adenocarcinoma	0/0	1/5	2/3	2/3	0/0	5 (45.4%)
Squamous CC	0/0	0/5	0/2	3/4	1/2	4 (30.7%)
Small CC	0/0	0/2	1/3	0/0	0/0	1 (20%)
Total of HER2+	0	1	3	5	1	10 (34.4%)

sion of the subsequently added DAB chromogen resulted in formation of brown precipitation at the antigen site.

Hercep Test™ was interpreted as negative for HER-2 protein overexpression (0 and 1+ staining intensity), and positive for HER-2 protein overexpression (weakly positive 2+ staining intensity and strongly positive 3+ staining intensity).

Results

The mean age of the patients was 55.4 years, ranging from 41 to 72 years. Twenty-six patients were males and 3 patients were females. More tumours were found in the left lung (19 cases, 65.5%) than in the right lung (10 cases, 34.5%). In the patients who underwent surgical treatment, lobectomy and mediastinal lymphadenectomy were performed. Other patients received radiotherapy and/or chemotherapy, depending on clinical stage. The follow-up of the patients was from 3 to 24 months. In two patients, the outcome of surgery was fatal. Two patients died of advanced disease, and one died of cardiac failure. Table 1 shows the patients distribution according to the histological type and clinical stage of lung cancer.

Histological analysis revealed 11 cases of adenocarcinoma, 13 cases of squamous CC and 5 cases of small CC. The majority of patients had a clinical stage II disease (12 cases), 8 patients had stage IIIA, 7 patients IIIB, and two stage IV.

The overall incidence of HER-2 overexpression was 34.4% (10 of 29). Higher incidence was found in the patients with adenocarcinoma – 45.4% (5 of 11) (Figure 1), while in squamous CC patients, the incidence was 30.7% (4 of 13) (Figure 2), and in small CC patients, the incidence was 20% (1 of 5) (Figure 3) (Table 2).

There was no statistically significant difference given the age and gender.

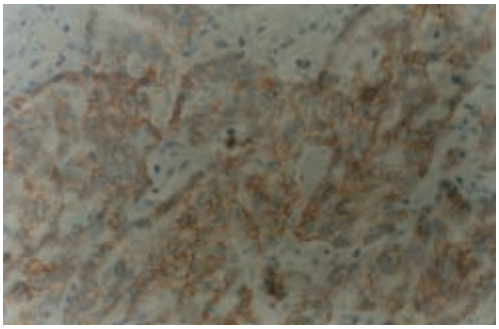

Figure 1. Positive immunostaining of HER-2 in pulmonary adenocarcinoma (DAKO Hercep Test ™).

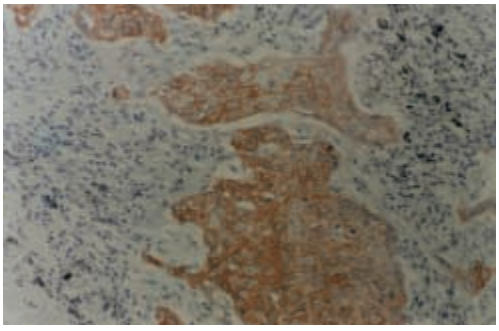

Figure 2. HER-2 overexpression in sqamous cell carcinoma of the lung (DAKO Hercep Test ™).

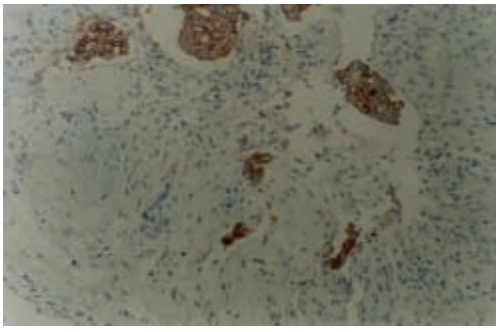

Figure 3. Positive immunostaining for HER-2 in small cell lung carcinoma (DAKO Hercep Test ™).

As shown in Table 2, HER-2 protein overexpression was more pronounced in the patients with advanced clinical stage: 4 of 5 patients (80%) with adenocarcinoma and HER-2 protein overexpression had stage IIIA and IIIB disease, and 3 of 4 patients (75%) with

squamous CC and HER-2 protein overexpression had stage IIIB disease, while of two patients with stage IV squamous CC, only one (50%) showed HER-2 protein overexpression. One patient with small CC and positive for HER-2 had in stage IIIA disease.

Discussion

Immunohistochemistry is a frequently used method for evaluating gene expression in tumours. However, a great variation in the interpretation of results has been noted. In the case of lung carcinoma, the positive rate of HER-2 expression ranged from 14 to 87.5%.[7] This variation is probably due to different methods, materials or subjective biases. There is also a possibility for heterogeneous expression of HER-2 in tumour cells. Hercep Test™ (DAKO) offers an objective and quantitative evaluation of immunohistochemical results. This is especially important when this immunohistochemically based marker is employed for assessing the therapy and prognosis.

In our study, the overall incidence of HER-2 overexpression was 34.4%, with a higher incidence found in the patients with adenocarcinoma – 45.4%. The patients with squamous CC overexpressed HER-2 in 30.7 % and those with small CC in 20%. These results are slightly higher than those presented in the review of Agus *et al*.[8] and could be due to the sensitivity of the antibody Hercep Test™ (DAKO).

There were many previous reports which have described the p185neu expression in lung cancer.[2-7] In some cases, the overexpression of HER-2/neu was shown to correlate also with the survival.[4,5] However, a limited number of cases, lack of a scoring system for HER-2/neu expression and conflicting results made the interpretation of these studies difficult. Harpole *et al*[9] suggested that, beside the other parameters (male, sex, presence of symptoms, tumour size, poor cell differentiation, vascular invasion, p53 expression and high Ki-67 index), the HER-2/neu expression could be an independent prognostic factor in the patients with stage I non-small cell lung cancer. They further propose that the outcome of the disease is the "dose response" of the additive effect of these parameters. It appeared that the disease progression could be dependent on several clinicopathologic parameters. The cause of early cancer deaths as well as the mechanism for the early relapse and metastasis remain to be determined.

Osaki *et al*[10] have shown that the patients with stage IIIB or T4 lung adenocarcinoma had an increased serum level of HER-2 and that it was correlated with the overexpression in tissue sections. Diez et al.[11], working on fresh samples of non-small cell lung cancer, did not find significant correlation between p185 level and TNM classification, but proved that shortened median time of tumour relapse was proportional to the rise of p185 in the tumour tissue.

The results presented in this paper demonstrate that the overexpression of HER-2/neu in the patients with lung carcinoma correlated with the advanced clinical stage. The follow-up of the patients in our study was short, so the determination of the relapse rate or disease-free period was limited. Two patients who died of the advanced disease had overexpression of HER-2 and short partial remission of 5 and 8 months.

In conclusion, our results are satisfactory and encourage us to continue this work in the follow-up study to evaluate HER-2/neu role as predictive and prognostic factor for the patients with lung cancer. However, the determination of this parameter is required in more patients and for longer period to confirm the value of HER-2 overexpression. The correlation with other oncoproteins is needed for the elucidation of a cause of early cancer death as well as of a mechanism for an early relapse and metastasising.

References

1. Cancer Registry in Macedonia. Skopje: Republic Institute for Health Protection; 1997. p. 83.

2. Pairolero PC, Williams DE, Bergstralh EJ, Piehler JM, Bernatz PE, Payne WS. Postsurgical stage I bronchogenic carcinoma: morbid implications of recurrent disease. *Ann Thorac Surg* 1984; **38:** 331-8.

3. Volm M, Effereth T, Mattern J. Oncoprotein (c-myc, c-erbB-1, c-erbB-2, c-fos) and suppressor gene product (p53) expression in squamous cell carcinomas of the lung. Clinical and biological correlations. *Anticancer Res* 1992; **12:** 11-20.

4. Weiner DB, Nordberg J, Robinson R, Nowell PC, Gazdar A, Greene MI, et al. Expression of the neu gene-encoded protein (P185neu) in human non-small cell carcinomas of the lung. *Cancer Res* 1990; **50:** 421-5.

5. Shi D, He G, Cao S, Pan W, Zhang HZ, Yu D, et al. Overexpression of the c-erbB-2/neu-encoded p185 protein in primary lung cancer. *Mol Carcinog* 1992; **5:** 213-8.

6. Hynes NE, Stern DF. The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim Biophys Acta* 1994; **1198:** 165-84.

7. Hsieh CC, Chow KC, Fahn HJ, Tsai CM, Li WY, Huang MH, et al. Prognostic significance of HER-2/neu overexpression in stage I adenocarcinoma of lung. *Ann Thorac Surg* 1998; **66:** 1163-4.

8. Agus DB, Bunn PA Jr, Franklin W, Garcia M, Ozols RF. HER-2/neu as a therapeutic target in non-small cell lung cancer, prostate cancer, and ovarian cancer. *Semin Oncol* 2000; **27:** 53-63.

9. Harpole DH, Herndon JE II, Wolfe WG, Iglehart JD, Marks JR. A prognostic model of recurrent and death in stage I non-small cell lung cancer utilizing presentation, histopathology and oncoprotein expression. *Caner Res* 1995; **55:** 51-6.

10. Osaki T, Mitsudomi T, Oyama T, Nakanishi R, Yasumoto K. Serum level and tissue expression of c-erbB-2 protein in lung adenocarcinoma. *Ches.* 1995;**108**: 157-62.

11. Diez M, Pollan M, Maestro M, Torres A, Ortega D, Gomez A, et al. Prediction of recurrence by quantification of p185-neu protein in non-small cell lung cancer tissue. *Br J Cancer* 1997; **75:** 684-9.