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research article

Prognostic value of immunohistochemical expression of HER-2/neu in patients with lung carcinoma

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Background. *The amplification and the overexpression of the Her-2/neu gene have been shown in certain human tumours and are postulated to be important in human carcinogenesis. In this study we evaluated the expression of HER-2/neu gene in patients with lung carcinoma (LC) and assessed its prognostic significance.*

Patients and methods. *HER-2/neu expression was determined in 127 LC patients using immunohistochemistry (IHC) performed on paraffin-embedded section – Hercep Test™ (DAKO).*

Results. *The overall HER-2/neu expression was seen in 36 (28.35%) of 127 LC patients. According to the histological type, HER-2/neu overexpression was detected in 12 patients with adenocarcinomas (60%), in 19 patients with squamous cell carcinomas (31.14%), in 4 patients with small cell-lung carcinomas (10%) and in 1 patient with other carcinomas (16.66%). Only in patients with small cell-lung carcinomas HER-2/neu overexpression was in correlation with the stage of the disease ($p<0.001$). The patients with HER-2/neu positive expression were associated with a significantly shorter survival compared with those who were HER-2/neu negative (log rank, $p<0.002$).*

Conclusions. *These observations suggest that HER-2/neu positivity may serve as a prognostic indicator in patients with LC. HER-2/neu plays a role in identifying patients at risk for the shortened survival, who may benefit from a more aggressive therapy.*

Key words: HER-2/neu, lung cancer; immunohistochemisty; survival

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Introduction

Lung cancer is the leading cause of cancer death worldwide with a still increasing incidence.[1,2] Despite new chemotherapeutic

drugs, improvement in surgical techniques, histological classification, and staging procedures, lung cancer survival has not greatly improved over the past 20 years. The cure rate remains less than 15%.[3] Recent advances in biology and molecular biology identified the relationship between specific gene alterations and clinical behaviour of lung cancers. A number of studies have been performed to assess the prognostic role of tumour-suppressor genes and proto-oncogenes.

HER-2/neu (also known as c-erbB2) oncogene is the second member of the epidermal growth factor of the receptor family.[4] The HER-2/neu dominant gene is localized in normal human cells as a singular copy on the long arm (q21) of chromosome 17.[5] This gene codes for a 185-kDa receptor-type tyrosine protein kinases (p185neu or c-erbB2), similar to EGF-R.[6-8] This 1255 aminoacid transmembrane glycoprotein is composed of there domains: extracellular factor-binding domain, transmembrane domain and intracellular domain with a tyrosine kinase activity.[9] When an EGF-like ligand (there is no known specific ligand for HER-2/neu) binds to a receptor of the EGF-R family, (HER-1, 3 or 4) there is a heterodimerisation of this receptor with HER-2.[10] HER-2 is necessary for the regulation of the normal cell growth and differentiation, and can be associated with multiple signal transduction pathways.[11] The amplification of the HER-2 gene leads to an overexpression of the receptor and protein product p 185, which could be implicated in the development of many types of tumours. HER-2/neu is expressed in a wide variety of human epithelial malignancies, including breast, ovary, salivary gland, gastrointestinal tract, prostate, lung, kidney, liver.[12-18] This suggests that HER-2/neu overexpression probably plays a critical role in the development and progression of human cancers.

The aim of this study was to determine the correlation between HER-2/neu expression and clinicopathological features in patients with lung carcinoma and to assess its relation to the survival.

Patients and methods

Patients and tissue samples

Our study population consisted of 127 patients with lung carcinoma (LC) diagnosed between January 2004 and December 2006 in the Institute for Lung Diseases and Tuberculosis, Skopje, Macedonia. Patients included 115 men and 12 women. Their age ranged from 37 to 87 years old (mean, 58.49 ± 8.15); 80.10% of them were smokers.

The diagnosis of LC was established by the histological examination of tissue samples obtained during the bronchoscopy in 120 patients (94.48%) or by surgery in 7 patients (5.51%). All histological analysis of the tumour tissue and immunohistochemistry (ICH) were performed at the Institute for Pathology, Medical Faculty, Skopje. The histological type and degree of differentiation were assigned according to World Health Organization criteria.[19] Tumours included 40 small-cell lung carcinomas (SCLC) and 87 non-small-cell lung carcinomas (NSCLC) – 20 adenocarcinomas, 61 squamous cell carcinomas and 6 tumours assigned as "others" (bronchoalveolare, large cell carcinomas, nondifferentiated carcinomas). For the histological differentiation, well moderately and poorly differentiated tumours were graded as grade 1, 2 and 3, respectively.

According to the International Staging System for Lung Cancer,[20] 87 NSCLC patients were divided in: 3 patients with IB stage (3.44%), 1 patient with IIA stage (1.14%), 11 patients with stage IIB (12.64%), 22 patients with stage IIIA (25.28%), 34 patients with stage IIIB (39.08%) and 16 pa-

tients with stage IV (18.39%). The patients with SCLC were staging in 23 patients with limited diseases (57.5%) and 17 patients with extensive diseases (42.5%).

One hundred and twenty patients were treated with chemotherapy and/or radiotherapy at the Institute for Radiotherapy and Oncology, Medical Faculty, Skopje. Seven patients underwent surgery first, and after that they were treated with radiotherapy. All patients were followed up regularly in a time frame of 2 to 3 months. The patients were followed up from 1 to 48 months (median 24 months). At the time of the last follow-up 114 patients (89.76%) had died and 13 patients (10.23%) were still alive. The survival time was calculated from the date of histological diagnosis to the date of death or last follow-up.

Immunohistochemistry

HER-2/neu oncogene expression was determined by immnunohistochemical staining with Hercep Test^{tm} (DAKO, Copenhagen, Denmark). After bronchoscopy or surgery, fresh tumour tissue specimens were immediately formalin fixed. Section 4 μm thick were cut from tissue blocks, placed on glass slides and exposed to xylene 3 times for 5 min each. Tissues were hydrated in decreasing concentrations of ethanol (100%, 95% and 70% for 2min each) and rinsed in distilled water. Slides were washed in PBS (Phosphate Buffer Saline) for 20 min. After excess liquid was blotted off, slides were heated at 120°C in 0.01 M citrate buffer (pH 6.0) to expose the HER2 protein antigen. The endogene peroxidase activity was blocked by Peroxidase Blocking Reagent for 5 min. The rabbit antihuman HER2 protein polyclonal antibody was applied for 2 h at room temperature. Slides were rinsed with PBS, and peroxidase-conjugated antirabbit antibody was added for 30 min at room temperature. Diaminobenzidine solution was added to achieve specific staining. Slides were counterstained with hematoxylin.

The evaluation of HER-2/neu immunoreactivity was performed according to the DAKO protocol for the Hercept Test^{tm}, with minor modifications. Only membrane staining was considered positive, whereas cytoplasmatic staining was considered nonspecific. Immunostaging was classified as follows: score 0: no staining at all or membrane staining in <10% neoplastic cells; score 1+: a faint/barely appreciable membrane staining is detected in >10% of the tumour cells; the cells are only stained in part of their membrane; score 2+: a with weak to moderate complete membrane staining is observed in >10% of the tumour cells; score 3+: a strong immunoreactivity of the entire membrane is observed in >10% of the tumours cells. Tumours classified as 0 or 1+ were considered "negative", and those scored 2+ or 3+ were classified as "positive".

Statistical Analysis

Statistical significances of the relationship between clinicopathological data and IHC were assessed by a chi-square test. The Fisher's exact test was used when the frequency of a cell in a 2 x 2 table was <5. The overall survival rate was calculated using the Kaplan-Meier method. The difference was considered to be statistically significant at $p<0.05$.

Results

Figure 1 shows the staining of HER-2/neu in adenocarcinoma using immunohistochemical methods. The dark brown colour denotes positive staining located predominantly at the cell membrane.

Quantitative data are shown in Table 1, which summarizes expressions of HER-2/neu

Table 1. The relationship between the expression of the HER-2/neu and clinicopathological features in patients with lung cancer

Features (n)	HER-2/neu + n (%)	p Value
Sex		
Male (115)	34 (29.56%)	
Female (12)	2 (16.66%)	NS
Age		
≤ 65 (90)	25 (27.77%)	
> 65 (37)	11 (29.72%)	NS
Smoking history		
Smoker (103)	27 (26.21%)	
Nonsmoker (24)	9 (37.5%)	NS
Performans status		
WHO 0 (27)	2 (7.40%)	
WHO 1 (79)	26 (32.91%)	
WHO 2 (11)	2 (18.18%)	
WHO 3 (9)	6 (66.66%)	
WHO 4 (1)	0 (0%)	p<0.006
Tumour type		
SCLC (40)	4 (10.0 %)	
NSCLC (87)	32 (36.78 %)	p<0.001
Differentiation		
Well (3)	2 (66.66 %)	
Moderate (55)	14 (25.45 %)	
Poorly (69)	20 (28.98 %)	NS
Stage (NSCLC)		
I B (3)	0 (0 %)	
II A (1)	0 (0%)	
II B (11)	4 (36.36 %)	
III A (22)	7 (31.81 %)	
III B (34)	11 (32.35 %)	
IV (16)	10 (62.5 %)	NS
Stage (SCLC)		
Limited (23)	0 (0 %)	
Extensive (17)	4 (23.52 %)	p<0.001
Total (127)	36 (28.35%)	

Note: statistical analysis was performed using x^2 test and Fisher exact test

in 127 LC patients with different clinicopathological features.

HER-2/neu immunoreactivity (3+/2+) was detected in 36 of 127 lung cancers cases (28.35%). Fifteen tumours (11.81%) showed strong HER-2/neu immunoreactivity (3+), while twenty one (16.54%) tumours were moderately immunoreactive (2+). Ninety-one cases (71.65 %) were considered negative; 81 cases were classified as score 0 (63.78 %) and 10 cases as score 1+ (7.87%).

As seen in Table 1, no statistically significant correlation was found between the frequency of HER-2/neu protein product expression and some clinicopathological

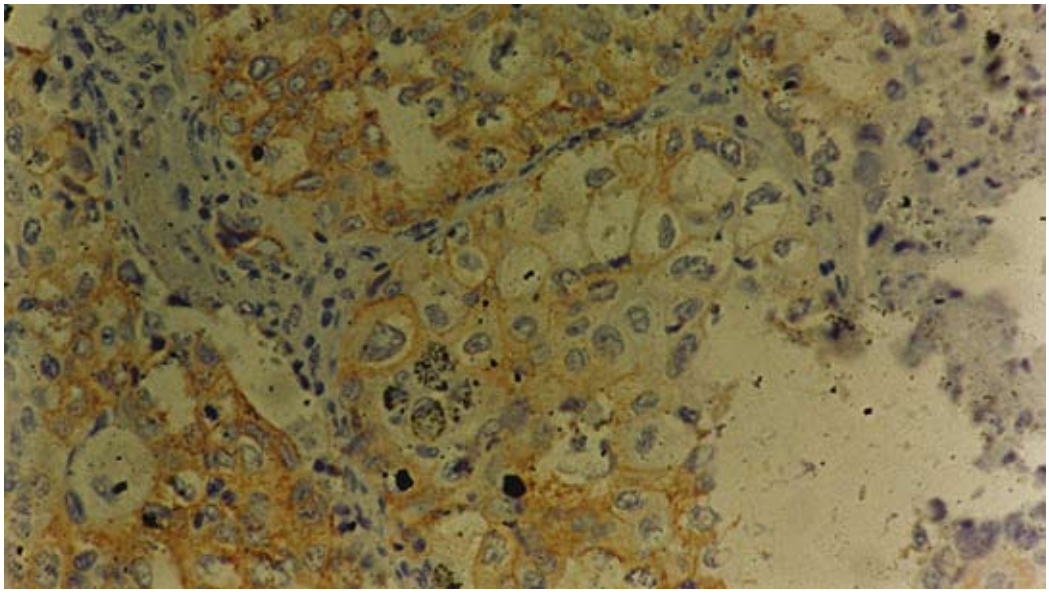

Figure 1. Immunohistochemical staining of HER-2/neu overexpression in lung adenocarcinoma (strong staining 3+).

features under the study including sex, age, smoking history and tumour differentiation (well, moderately and poorly). We found the correlation between HER-2/neu expression and performance status (x^2=14.1; p<0.006). We also obtained the significant differences in HER-2/neu expression between SCLC and NSCLC (p<0.001). Among the NSCLC's HER-2/neu expression was found in 12 of 20 (60%) adenocarcinomas, in 19 of 61 (31.14%) squamous cell carcinomas, and in 1 of 6 (16.66%) "other" carcinomas (Table 2). The difference in the expression rate between the adenocarcinomas and the other groups was highly significant (x^2=17.13; p<0.0001). Eight patients (66%) with HER-2/neu positive adenocarcinomas showed strong immunoreactivity (3+).

In NSCLC patients HER-2/neu expression was not in correlation with the stage of the diseases. But, there was a significant difference in HER-2/neu expression between SCLC patients with limited and extensive diseases (Fisher exact test p<0.001).

We found that the expression of HER-2/neu protein was associated with a significant short survival in LC patients. The 24-months survival rates of patients with HER-2/neu overexpresssion and those without were 21.97% and 2.77%, respectively (Figure 2) with a statistical significant difference (log-rank Test = – 3.06; p=0.002).

Discussion

Since HER-2/neu proto-oncogene has been originally identified, its overexpression was detected in various types of cancers, first of all in those with epithelial origin.[21] HER-2/neu expression is associated mainly with high-grade breast carcinoma and considered an important prognostic factor for adverse outcome in node positive breast cancer.[13] In lung cancer studies, HER-2/neu expression varies depending on the histological classification of the tumours.[4,10,22-24] Her-2/neu expression was reported in 13-80% in adenocarcinomas,[17,22,24] in 2-45% in squamous cell carcinomas,[15,16,18] in 0-20% in large-cell carcinomas[23] and in 13-31% in small cell lung carcinomas.[4,25,26]

Table 2. HER-2/neu expression in different histological type of lung cancer

Histological type	No of cases	**Level of expression***				
		+++	**++**	**+**	**–**	**% positive**
SCLC	40	2	2	2	34	10.0
Squamous cell cacinoma	61	5	14	3	39	31.14
Adenocarcinoma	20	8	4	5	3	60.0
Others	6	0	1	0	5	16.6

* –: no detectable HER-2/neu expression; +, ++, +++: faint, weak and strong expression, respectively

We observed HER-2/neu overexpression in 36 of 127 patients with lung carcinoma (28.25%). The overexpression was more frequent in NSCLC (36.78 %) than in the SCLC (10.0%). The patients with adenocarcinoma showed the highest incidence of positive findings (60%), with the biggest number of strong reactivity patterns (53.33%). This observation concurs with the findings of Shi *et al.*, Harpole *et al.*, Pellegriniet *et al.*, Hsieh *et al.* and others.[8,17,27,28]

The techniques used to detect HER-2/neu expression might be one of the potential sources of biases. HER-2/neu protein overexpression is most often measured by IHC using one of several monoclonal antibodies, and the gene expression is measured most often by fluorescence in situ hybridization (FISH) for clinical studies.[5] Immunohistochemical results can vary according to the primary used antibody, dilution of the antibody and tissue conservation. Moreover, this technique is semiquantitative and standards for positive or negative specimen vary between studies making difficult the direct comparison of studies.

In this study we did not find any correlation between HER-2/neu overexpression and the pathologic stage of disease in patients with NSCLC. Even our results showed very low HER-2/neu overexpression in patients with SCLC, we found significant differences of HER-2/neu overexpression between SCLC patients with limited and extensive diseases: HER-2/neu overexpression was seen only in patients with the extensive disease (Fisher exact test $p<0.05$). Tateishi *et al.*[22] reported a higher positivity for HER-2/neu in Stage III-IV disease with a poor influence on the prognosis. Similarly, Osaki *et al.*[29] reported increased serum levels of HER-2/neu in Stage IIIB cases. However, the comparison among different studies is difficult due to a consistent lack of balance in the histotype and the stage distribution from one study to another.

Although HER-2/neu protein expression is widely studied in lung carcinoma, its prognostic role remains uncertain. Even several investigators reported that HER-2/neu immunostaining adversely affects the prognosis and the survival in LC patients, especially NSCLC patients,[17,18,22,23,30] some large studies did not find any prognostic implication for HER-2/neu overexpression.[31-33] There are many studies showing adverse outcomes among patients with breast and ovarian cancers overexpressing this oncogene. Besides, the overall survival rate and time to relapse for those patients with HER-2/neu overexpression is shorter than those lacking the overexpression.[13,21,34] Our results show that patients with HER-2/neu positive tumours had a significantly decreased survival opposite to patients with HER-2/neu negative tumours. After 24 months, only one patient from the group who were HER-2/neu positive was still alive, in comparison with 20 patients from the group who were HER-2/neu negative.

There are studies which have shown that HER-2/neu overexpression is associated with intrinsic chemoresistance of NSCLC.[35]

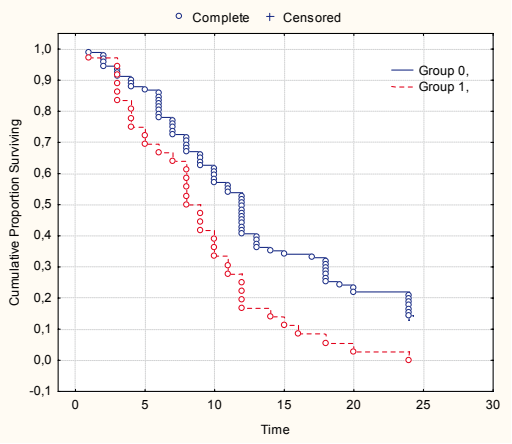

Figure 2. 24-month survival of a patient with lung cancer as a function of absence (solid line) or presence (dashed line) of HER-2/neu overexpression.

Thus, HER-2/neu overexpression may play a role as a predictive factor of response to therapy in patients with LC, who may be candidates for adjuvant therapeutic modalities and the gene therapy possibly improving the overall survival.

Conclusions

In conclusion, HER-2/neu expression detected in LC is a negative predictor of the survival. Immunohistochemical stainig of Her-2/neu may aid in defining a subpopulation of patients with LC whose tumours may behave more aggressively. In addition, focused adjuvant therapeutic modalities and gene therapies might improve the overall survival in these patients expressing the poor prognostic immunohistochemical marker.

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