ADJUVANT CHEMOTHERAPY EFFECTIVENESS FOR I–II STAGE NON-SMALL CELL LUNG CANCER (NSCLC) PATIENTS DEPENDING ON THE TUMOR MOLECULAR PROPERTIES

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Abstract

Adjuvant chemotherapy use on early stages non-small cell lung cancer patients remains the debatable issue. For individualization purpose of adjuvant chemotherapy administration it is possible to use molecular prognostic/predictive markers. The goal of our study was to investigate the adjuvant chemotherapy effectiveness on early stage NSCLC patients depending on the various molecular markers expression in primary tumor. Included in research are 254 patients with I–II stage non-small cell lung cancer (NSCLC) that received institutional treatment in the department of thoracic surgery of Zaporizhyzhya Regional Clinical Oncologic Dispensary starting from June of 2008 up to December of 2012. For adjuvant chemotherapy administration individualization purpose the following molecular markers were used: Ki-67, CD31/CD34, Pan-Cytokeratin, Her-2/neu, p53, E-cadherin, EGFR. Low Ki-67, CD31/CD34, p53 expression level is a marker of adjuvant chemotherapy negative influence on early stage NSCLC patients survival rate. Alternatively, at high expression level of the said markers the adjuvant chemotherapy administration significantly improves examined patients survival rate. Pan-Cytokeratin indicates the presence of micrometastases in the lung root lymph nodes, and those are such patients, whose survival rate is improved by adjuvant chemotherapy conduction. Her-2/neu is the marker of tumor chemoresistance, more specifically, for patients with high expression level of such marker conducting of adjuvant chemotherapy is not appropriate, because this type of treatment does not improve survival at indicated marker characteristics. At the same time, low level of Her-2/neu expression is a marker of chemosensivity and adjuvant chemotherapy conduction on such patients improves their survival rate. Significant predictive connection of E-cadherin EGFR expression level, high Her-2/neu expression and the absence of micrometastases is not established.

Keywords: Adjuvant chemotherapy, non-small cell lung cancer, Ki-67, survival rate

Introduction:

Surgery remains the basic method of treatment for patients with early (I-II) stages of non-small cell lung cancer (NSCLC). However, for 50-60% of patients the disease progression occurs after surgical interference. In order to improve outcomes of patients with early stages of NSCLC the use of adjuvant chemotherapy on the basis of platinum-based drugs is possible. Adjuvant chemotherapy effectiveness for this category of patients is still debated. A number of studies found no improvement in survival rate in patients after conducting of adjuvant chemotherapy [5,6,8]. According to other authors' data adjuvant chemotherapy is effective for patients with II and IIIA stages of NSCLC [1,2,7,9].

Perhaps, the difference of the received data is due to the heterogeneity of patients involved in the research. Today it is known that NSCLC patients form the heterogeneous

group, even within the same stage, which is associated with tumor molecular characteristics [3,4]. Molecular markers may be used in order to separate subgroups.

Materials and Methods: 254 patients with I–II stage non-small cell lung cancer (NSCLC) that received institutional treatment in the department of thoracic surgery of Zaporizhyzhya Regional Clinical Oncologic Dispensary are included in research. Patients' average age amounts to 60,7 years (95% CI 59,7-61,7). All patients underwent surgical treatment in the amount of lobectomy or pneumonectomy. Adjuvant chemotherapy was conducted for 52 patients with I stage of NSCLC and 49 patients with II stage of the disease. Adjuvant chemotherapy conduction for patients with I stage of NSCLC is not the care standard, in connection with which after explaining of all the possible positive and negative adjuvant chemotherapy effects, 119 patients refused additional therapy offer. Part of the patients with stage II (n=34) did not receive adjuvant chemotherapy due to comorbidity, or rejection of this type of treatment. Adjuvant chemotherapy involved 2-3 courses of platinum-based chemotherapy (80mg/m2 of cisplatin for 1 day, 120 mg/m2 of etoposide from the 1st to the 3rd day, with 21-day interval between the courses). Chemotherapy treatment was initiated within 21 days after surgery.

For adjuvant chemotherapy administration individualization purpose the following molecular markers were used: Ki-67, CD31/CD34, Pan-Cytokeratin, Her-2/neu, p53, E-cadherin, EGFR.

X^2 criterion and Pearson's chi-squared test were used for patients with adjuvant chemotherapy and without additional treatment in order to evaluate the connection between various clinical and morphological features. The survival rate was estimated using the Kaplan-Mayer function. Survival rate difference of individual groups was examined using log-rank criterion. The significance level was defined as $p<0.05$. Statistica 6.0. Software package was used for material statistical processing.

Results of research: One of the most important tumor properties is its proliferative activity. The adjuvant chemotherapy effectiveness was analyzed for patients with low and high proliferative activity. For patients with low proliferative activity median survival after conducting of adjuvant chemotherapy amounted to 25 ± 3,7 months, while for patients for whom the additional treatment was not conducted the survival rate was significantly higher ($p<0,001$) - median survival was not reached (Fig. 1.). In other words for patients with low proliferation level the conducting of adjuvant chemotherapy significantly worsens their survival rate.

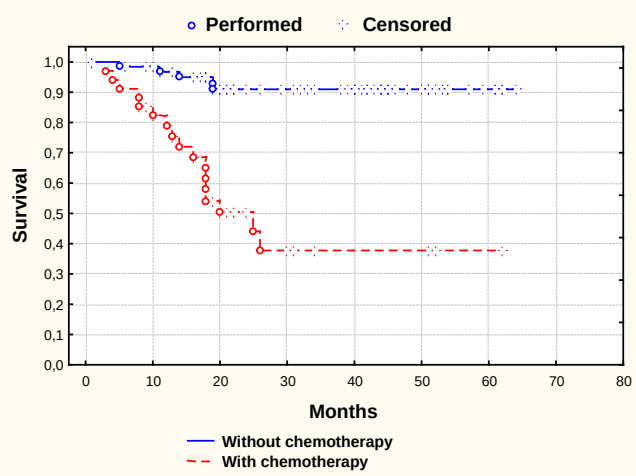

Fig. 1. Patients' survival rate at early NSCLC stage with low proliferative activity level depending on adjuvant chemotherapy administration

However, for patients with high proliferation in primary tumor, who had received adjuvant chemotherapy , survival rate considerably increased ($p<0,001$, Fig. 2.).

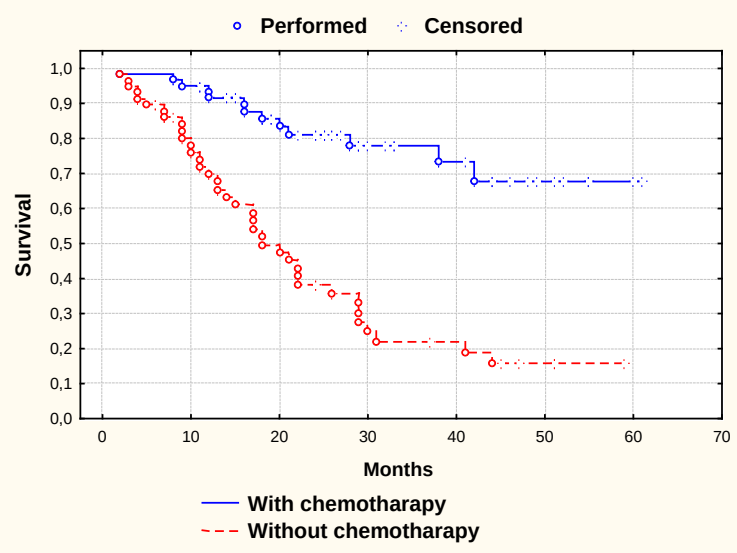

Fig. 2. Patients' survival rate at early NSCLC stage with high proliferative activity level depending on adjuvant chemotherapy administration (institution)

Thus, for patients with high proliferation index, which had received adjuvant chemotherapy, the median survival was not achieved. The 75th percentile of survival equals to 38 ± 11,4 months. The survival rate of patients that had not received additional chemotherapy treatment was almost two times less. In this case 75th percentile of survival rate amounted to 11,0 ± 1,8 months. Therefore, conducting of adjuvant chemotherapy is only necessary for patients with high malignant neoplasms proliferative activity.

Another molecular factor that was analyzed was the tumor microvessels density (MD), which was analyzed with the help of CD31/CD34 expression. For patients with low tumor MD that had received adjuvant chemotherapy in the postoperative period, median survival amounted to 42.0 months, whereas for patients with a similar molecular characteristics of the tumor that had not received adjuvant chemotherapy this indicator was not achieved. Survival rate in two groups was different (p=0,005, Fig. 3).

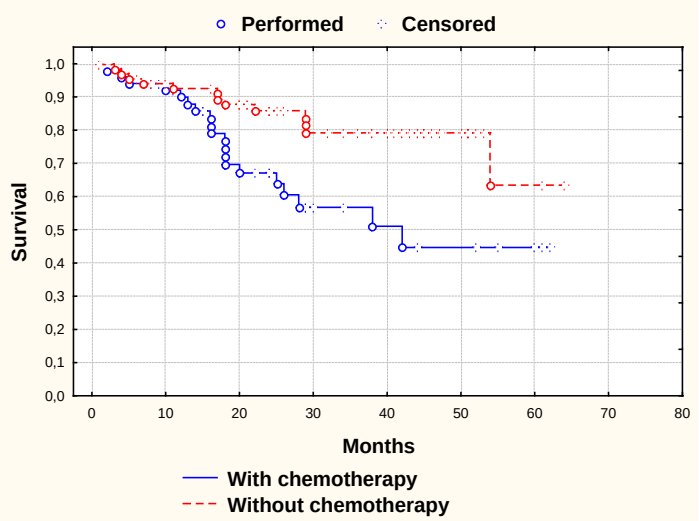

Fig. 3. Patients' survival rate at early NSCLC stage with low microvessels density level depending on adjuvant chemotherapy administration

For patients with high tumor microvessels density level the conducting of adjuvant chemotherapy conversely improved distant survival rate (p=0,003, Fig. 4.). Thus, the median survival of patients with high primary tumor MD after further postoperative chemotherapy was not achieved, while the median survival of patients without adjuvant chemotherapy was 30.0 months.

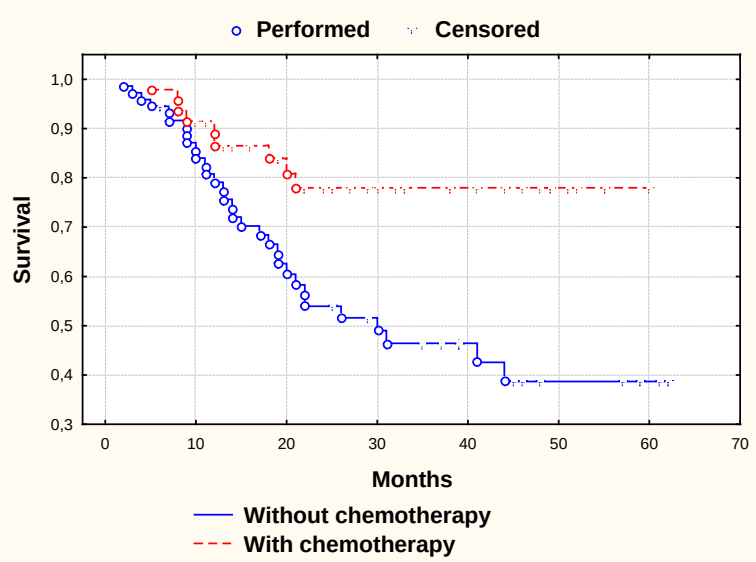

Fig. 4. Patients' survival rate at early NSCLC stage with high microvessels density level depending on adjuvant chemotherapy administration.

Thus, there is a need of adjuvant chemotherapy conducting only in cases with high primary tumor MD and it is of no use when MD is low.

Another factor except for the proliferative activity and tumor MD that was analyzed is the existence of micrometastases in the lung root lymph nodes, which was assessed with the help of Pan-Cytokeratin expression. In the absence of micrometastases the survival rate of patients did not change after adjuvant chemotherapy appointment (p=0,884). Thus, the median survival was not achieved within the group of patients without micrometastases and without adjuvant chemotherapy conducting and for patients without micrometastases with conducting of adjuvant chemotherapy. This indicates adjuvant chemotherapy efficacy lack for patients without micrometastases. Additional chemotherapy effectiveness assessment for patients with micrometastases on the contrary showed a significant difference in survival rate of patients with and without adjuvant chemotherapy conducting (p=0,005, Fig. 5). Thus, the median survival rate of patients with micrometastases in the lung root lymph nodes after conducting of adjuvant chemotherapy was not achieved, while the median survival of patients without additional treatment was 13,0±6,3 months.

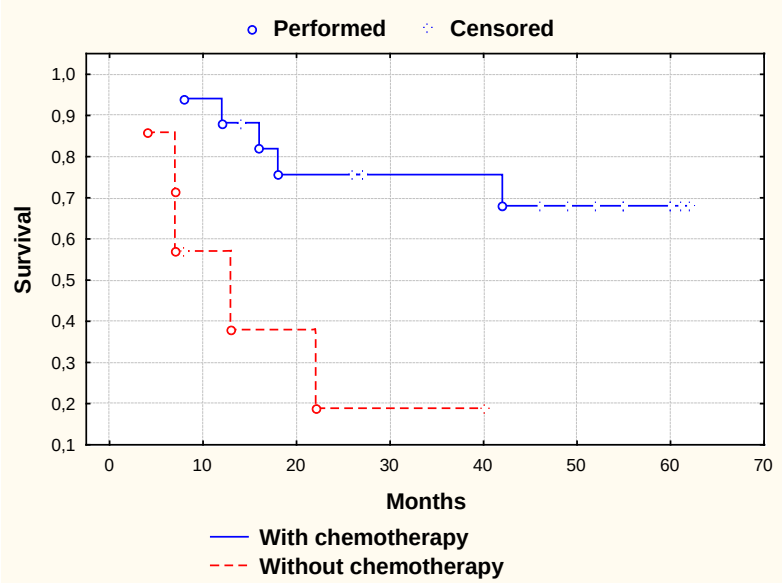

Fig. 5. Patients' survival rate at early NSCLC stage with micrometastases in the lymph nodes of root of the lung depending on adjuvant chemotherapy administration.

In recent years, increasing attention was paid to the status of the epidermal growth factor receptor in primary lung tumors. In our research, we have examined the EGFR and Her-2/neu expression, and also analyzed the adjuvant chemotherapy effectiveness depending on indicated markers expression level.

Patients with low EGFR expression level: median survival is not achieved by patients without further treatment in the postoperative period and as well as by patients with conducting of adjuvant chemotherapy. The difference in the survival of two groups was not statistically significant (p=0,877). Similar results were obtained for patients with high EGFR expression level. The median survival rate of patients with adjuvant chemotherapy amounted to 25.0 months, and patients without adjuvant chemotherapy - 30.0 months. There is no difference in survival rate (p=0,560). Therefore, the expression of EGFR is not a predictive factor for adjuvant chemotherapy efficiency for patients with early-stage NSCLC.

The studied patients in terms of Her-2/neu predictive role, were also divided into groups of patients with low and high levels of marker expression. 75th percentile survival rate was not achieved for patients with low Her-2/neu expression after conducting of additional treatment. At the same time, patients with low expression and no adjuvant chemotherapy had a significantly lower survival rate - 75th percentile survival amounted to 41 months (p = 0.019, Fig. 6).

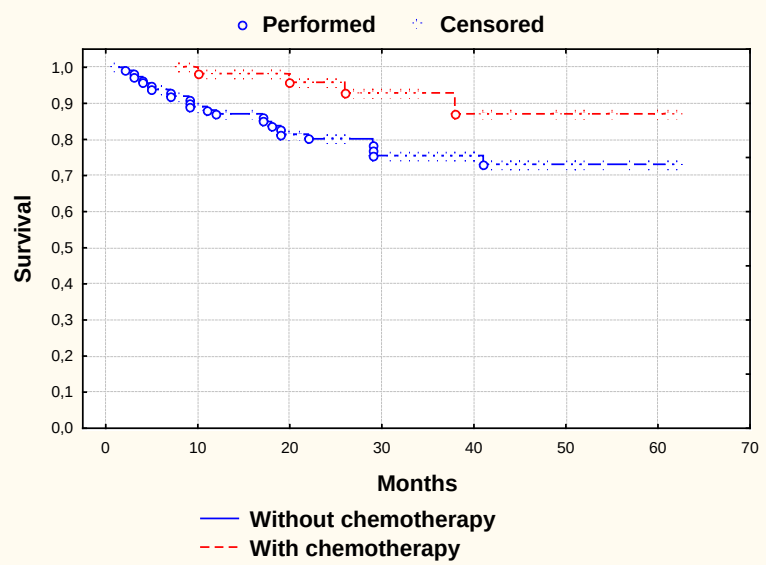

Fig. 6. Patients' survival rate at early NSCLC stage with low Her-2/neu expression level depending on adjuvant chemotherapy administration

For patients with high Her-2/neu expression level adjuvant chemotherapy conduction impact was not observed (p=0,880). The median survival of patients with adjuvant chemotherapy amounted to 18.0 months, and in the absence of additional postoperative chemotherapy - 21.0 months.

Thus, high level of Her-2/neu indicates tumor chemoresistance.

The following researched the molecular marker is p53 apoptosis marker. Apoptosis is one of tissue important properties, including the tumor tissue. In order to conduct the research of adjuvant chemotherapy predictive value, all patients included in the research were divided into groups of patients with high and low levels of p53 expression, and then the survival rate was determined in each group, depending on adjuvant chemotherapy conducting.

As shown in Figure 7 significant difference in the survival rate of patients with low p53 expression was marked, moreover, patients who had received chemotherapy had significantly worse survival rate (median survival - 26.0 months) compared to the patients who had not received adjuvant chemotherapy - median survival was not achieved. The difference in survival is statistically significant (p=0,008).

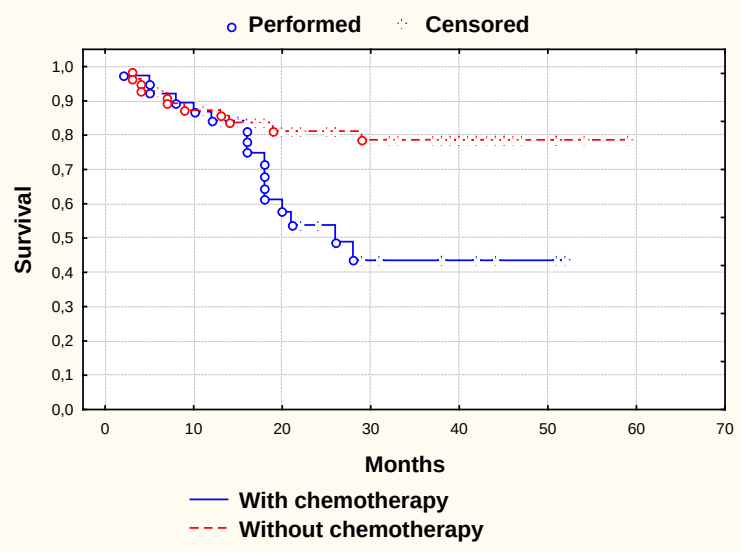

Fig. 6. Patients' survival rate at early NSCLC stage with low p53 expression level depending on adjuvant chemotherapy administration

In the presence of early-stage NSCLC patients high p53 expression we have also detected a significant difference in survival rate for patients who had received adjuvant chemotherapy and patients that had not received additional treatment ($p = 0.002$). In Fig. 8. it is shown that the median survival of patients with high p53 expression and conducted adjuvant chemotherapy was not achieved. For patients with p53 overexpression and without additional treatment the median survival amounted to 31,0 ± 7,9 months.

Thus, adjuvant chemotherapy conducting for patients with p53 overexpression has a significant positive effect on survival of patients with stage I-II NSCLC. As opposed to this the conducting of additional postoperative chemotherapy for patients with low expression significantly worsensed their survival rate.

Last molecular marker that has been analyzed is E-cadherin - cell adhesion marker. For patients with low E-cadherin expression level and that had not received adjuvant chemotherapy the median survival was not achieved. While after the conducting of adjuvant chemotherapy it equaled 20.0 months, this difference is not statistically significant ($p = 0.101$). At the same time, survival rate of patients with high E-cadherin expression level was also not improved by adjuvant chemotherapy ($p = 0.452$). Median survival was not achieved by both: patients after conducting of adjuvant chemotherapy and patients that had not received additional postoperative treatment.

Therefore, out of all analyzed markers the following ones are predicatively significant: Ki-67, CD31/CD34, Her-2/neu, p53, Pan-Cytokeratin.

Ki-67, CD31/CD34, p53 low expression levels are adjuvant chemotherapy negative impact marker on patients with early-stage NSCLC survival rate. On the contrary, at these markers high expression levels the adjuvant chemotherapy appointment significantly improves the studied patients' survival rate. Pan-Cytokeratin indicates the micrometastases presence the lymph nodes of root of the lung and for these types of patients the conducting of adjuvant chemotherapy improves the survival rate. Her-2/neu is a marker of tumor chemoresistance, and for patients with high expression level of this marker, in particular, the conducting of adjuvant chemotherapy is not reasonable, since this type of treatment does not improve survival rate under the specified marker characteristic. At the same time, low Her-2/neu expression level is a marker of chemo sensitivity and the conducting of adjuvant chemotherapy for such patients improves their survival rate.

Patients' median survival analysis with different molecular markers expression and conducting or absence of adjuvant chemotherapy was carried out in order to confirm the obtained data (Table 1).

Table 1 I–II stage NSCLC patients survival rate depending on the molecular markers expression level and prescribed treatment

Marker	Patients number	Median survival	p
Pan-Cytokeratin IHC (immune histochemical study) (-)			
Observations	104	NA*	0,884
Adjuvant chemotherapy	52	NA *	
Pan-Cytokeratin IHC (+)			
Observations	7	13,0±6,3	0,005
Adjuvant chemotherapy	17	NA *	
E-cadherin IHC (-)			
Observations	33	NA *	0,101
Adjuvant chemotherapy	29	20,0±4,0	
E-cadherin IHC (-)			
Observations	74	NA *	0,452
Adjuvant chemotherapy	33	NA *	
EGFR IHC (-)			
Observations	60	NA *	0,877
Adjuvant chemotherapy	42	NA *	
EGFR IHC (+)			
Observations	66	30,0±10,7	0,560
Adjuvant chemotherapy	31	25,0±6,3	
CD31/CD34 IHC (-)			
Observations	70	NA **	0,005
Adjuvant chemotherapy	51	42,0±11,5	
CD31/CD34 IHC (+)			
Observations	73	30,0±8,9	0,003
Adjuvant chemotherapy	47	NA *	
p53 IHC (-)			
Observations	56	NA *	0,008
Adjuvant chemotherapy	38	26,0±5,3	
p53 IHC (+)			
Observations	84	31,0±7,9	0,002
Adjuvant chemotherapy	42	NA *	
Her-2-neu IHC (-)			
Observations	115	NA *	0,019
Adjuvant chemotherapy	58	NA *	
Her-2-neu IHC (+)			
Observations	30	21,0±2,5	0,88
Adjuvant chemotherapy	40	18,0±1,8	
Ki-67 IHC (-)			
Observations	63	NA *	p<0,001
Adjuvant chemotherapy	34	25,0±3,7	
Ki-67 IHC (+)			
Observations	58	18,0±2,3	p<0,001
Adjuvant chemotherapy	61	NA	

* Note: NA - not achieved

As is evident from table data, the significant predictive connection of E-cadherin, EGFR expression level, high Her-2/neu expression and micrometastases absence was not detected. Further data meets the abovementioned and once again confirms the results.

Conclusion

1. Of all analyzed markers the following are predicatively important: Ki-67, CD31/CD34, Her-2/neu, p53 and Pan-Cytokeratin.
2. Ki-67, CD31/CD34, p53 low expression level is adjuvant chemotherapy negative impact marker for early stage NSCLC patients survival rate and vice versa at these markers

high expression levels the adjuvant chemotherapy appointment significantly improves survival rates.

3. Pan-Cytokeratin expression presence indicates micrometastases in lymph nodes of root of the lung, and for these patients the adjuvant chemotherapy appointment improves survival rates.

4. Her-2/neu is a marker of tumor chemoresistance. Thus, for patients with high expression level of this marker, in particular, the conducting of adjuvant chemotherapy is not reasonable, since it does not improve survival rate. At the same time, the low Her-2/neu expression is a marker of chemo sensitivity and the appointment of adjuvant chemotherapy improves such atients' survival rate significantly.

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