ORIGINAL RESEARCH

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Relationship Between Xanthine Oxidoreductase Activity and BRCA1 Levels in Patients with Stage IIIA and IIIB Non-Small Cell Lung Cancer Being Treated with Neoadjuvant Chemotherapy

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ABSTRACT Objective: Lung cancer is the leading cause of cancer-related death worldwide. Treatment in locally advanced non-small cell lung cancer (NSCLC) is heterogeneous. The cure rates after complete surgical resections are not as good as expected. A better understanding of the biology of NSCLC might allow the selection of appropriate treatment. Only a few studies have been carried out on the prognostic value of xanthine oxidoreductase (XOR) and BRCA1 in lung cancer. **Material and Methods:** In this study, 35 patients with stage IIIA and stage IIIB of NSCLC were included. They were operated in Baskent Ankara and Adana hospitals and received neoadjuvant chemotherapy. The regular follow-up of all the patients was done in Baskent University Medical Oncology, Thoracic Surgery Department. The clinical and histopathological parameters (age, gender, stage, smoking history, performance status, and neoadjuvant chemotherapy), along with the immunohistochemical study of BRCA1 and XOR staining, were examined, and correlated with survival outcomes. **Results:** Median overall survival time was reported as 38.5 months, and 5-year survival rate was 33%. The presence of BRCA1 was positively associated with shorter overall survival in stage III lung cancer patients, who were followed up with the neoadjuvant platinum-based chemotherapy regime ($p<0.05$). There was no relation between XOR activity and overall survival outcomes. **Conclusion:** BRCA1-positive status might be prognostic in patients with Stage IIIA and IIIB of NSCLC.

Keywords: BRCA1; NSCLC; prognosis, and XOR activity

The current standard therapeutic regimen for the people with locally-advanced non-small cell lung cancer (NSCLC) without a mutation is platinum-based double-agent chemotherapy. Their effectiveness against NSCL and systemic toxicity must be known in advance to utilize these drugs effectively.

Many biomarkers have emerged as prognostic and predictive markers for NSCLC. Among them, the common biomarkers are the epidermal growth factor receptor (EGFR), 5 endonuclease enzyme of nucleotide excision repair (ERCC1), Kirsten sarcoma virus, which is a proto-oncogene (K-ras), and regulator subunit of ribonucleotide reductase. Besides, there is huge evidence suggesting that there might be a prognosis between NSCLC and BRCA1, XOR.[1-3]

Many studies on the prognostic and predictive values of BRCA1 and XOR in patients with NSCLC have been carried out previously. However, very few of them have addressed the effect of neoadjuvant chemotherapy on pathological tissue markers.[4-8]

This study aimed to determine the role of XOR activity in NSCLC and show the association of

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BRCA1 and its prognosis. We also monitored the changes at the tissue level in the presence of these markers during neoadjuvant chemotherapy.

MATERIAL AND METHODS

The present study was carried out on thirty-five cases of NSCLC, stage-III. The subjects had a history of neoadjuvant chemotherapy at Başkent University Department of Oncology, between the years of 2006-2012. The patients' files were reviewed for the demographic factors of age, gender, stages of the disease at the time of diagnosis, administration of neoadjuvant chemotherapies, disease-free survival rate, and overall survival outcomes.

The mouse monoclonal antibody (MS110) and XOR ((DAKO Carpentaria California USA) were tested on patients during diagnosis and after the surgery.

Both antigens were stained with the antibodies and scored. The relations between the prognosis and characteristics of the disease, and treatment response were examined.

All the sections stained with HE and IHC were examined blindly in terms of histological grade, without knowing the previous results of IHC. In all the cases, surrounding lung tissue in the tumor and existing sections were evaluated separately for both of the primer antibodies. Nuclear staining was done for BRCA1 and cytoplasmic staining done for XOR.

Scoring for XOR: Staining density was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strongly positive), while the percentage of positive staining was scored as 0 (negative), 1 (1-9%), 2 (10-49%), and 3 (50%), where 0-1 was graded as decreased and 2-3 was graded as increased.

Scoring for BRCA: Staining density was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strongly positive). These patients were considered as BRCA1 (+) since their BRCA1 score was 3, and the patients were considered as BRCA1 (-) when their scores were lower than 3 (0, 1, and 2 graded as negative). The chemotherapy responses were analyzed by RECIST 1.0.

STATISTICAL ANALYSIS

Descriptive statistics were performed for the demographic and clinical features of the patients. Student's t-test or Mann-Whitney U test was performed to compare the numerical variables of the two independent groups while Chi-square test was performed to compare the groups. Kaplan-Meier analysis was performed to analyze the effect of the pre-determined factors on survival. The survival of different groups was compared using a log-rank test. Cox-regression test was used for the multivariate analysis of the factors associated with relapse-free survival and overall survival. The variables that had the value of $p<0.2$ in univariate analysis were included in the multivariable analysis. Statistical analyses were performed by SPSS, ver. 17.0 (SPSS Inc, Chicago, IL). The $p>0.05$ was considered statistically significant.

RESULTS

Thirty-five patients were included in the study. The median age was 60 among the range of 45-79. Twenty-nine (82.9%) patients were male, while 6 (17.1%) were female. ECOG PS status of the patients: Seven of them were noted as 0 (20%), twenty-four were noted as 1 (68.8%), while four were reported as 2 (11.4%). According to the histopathological examination of the patients, 16 patients (45.7%) were recorded as an adenocarcinoma, 15 (37.1%) were having squamous cell carcinoma, 2 (5.7%) were suffering from large cell carcinoma, and 2 (5.7%) of the patients were in the group that was not subclassified. Among them, 19 patients (54.3%) were at stage IIIA, and 16 (45.7%) were at stage IIIB. After neoadjuvant chemotherapy, 29 patients were in partial remission, 3 patients had stable disease, 2 patients had progressive disease, and 1 patient showed complete response. Afterward, relapse was observed in 27 patients. The median progression was calculated until the assessment at 24 months. Twenty-two patients were lost during follow-ups. The demographic data of the patients are summarized in Table 1.

The median follow-up time of the patient was 37.4 months, median survival was 38.5, and survival for 60 months was calculated as 33% (Figure 1).

TABLE 1: Patient characteristics.

Number of the patients	35
Age, Median	61 (38–71)
Gender	
Male	29 (82.9%)
Female	6 (17.1%)
Clinical stage	
IIIA	19 (54.3%)
IIIB	16 (45.7%)
Histology	
Adenocarcinoma	16 (45.7%)
Squamous cell carcinoma	15 (42.9%)
Large cell and others	4 (11.4%)
ECOG Performance	
0	7 (20%)
1	24 (68.6%)
2	4 (11.4%)

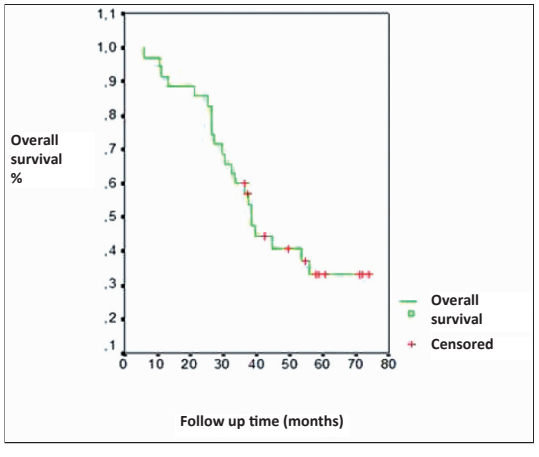

FIGURE 1: Overall survival curve.

Overall survival analysis indicated that those with the value of ECOG PS as 0 were more advantageous than those with ECOG value of 2 (p=0.0039) (Figure 2).

BRCA1 IHC interpretation of biopsy samples taken before chemotherapy of nine patients was positive and 26 patients showed a negative report. BRCA1 survival analysis indicates that there is a statistically significant difference between the two groups (p=0.047) (Figure 3). BRCA1 expression in operation blocks, obtained after neoadjuvant chemotherapy or radiotherapy did not change in any of the patients (Figure 4). After the operation, the relation between positivity in BRCA1 and survival was found to be statistically significant in the negative direction (p=0.047) (Figure 4). There was no statistical difference between histopathological investigation of the patients and their BRCA1 expression (Table 2).

On the other hand, when we looked at XOR activity, we found that XOR activity increased in the biopsy samples of 11 patients (31.4%) out of 35 while it decreased in the remaining 24 patients (66.6%).

The relation between XOR activity and overall survival was found to be at the borderline level of statistical significance (p=0.05) (Figure 5). After the neoadjuvant treatment, the IHC and XOR findings of two patients, which was positive earlier, changed to negative (Table 3). There was no change in negative patients. In the operated patient samples, 9 (25.7%) showed positive, and 26 (74.3%) showed negative

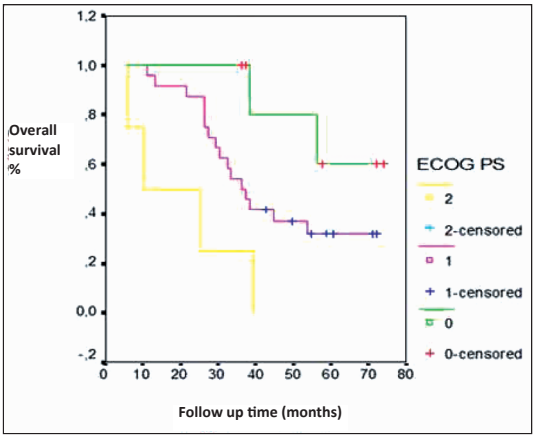

FIGURE 2: ECOG PS and overall survival (p=0.0039).

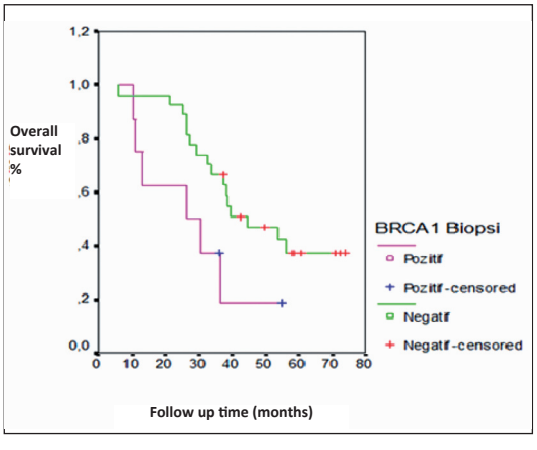

FIGURE 3: BRCA1 biopsy and overall survival curve (p=0.0476).

TABLE 2: Histopathology and BRCA1 relation.

	BRCA1 Biopsy		
	Negative	Positive	Total
Adenocarcinoma	12 (75%)	4 (25%)	P=0.941
Squamous cell carcinoma	11 (75%)	4 (25%)	
Large cell and others	3 (75%)	1 (25%)	
Total	26 (74,2%)	9 (25,19%)	

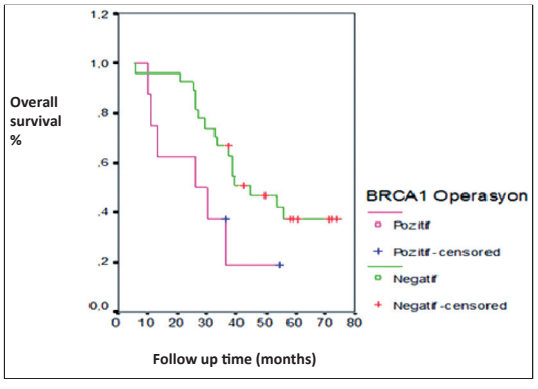

FIGURE 4: BRCA1 postoperation and overall survival curve (p=0.0476).

TABLE 3: Histopathology and XOR relation.

	XOR Biopsy		
	Negative	Positive	Total
Adenocarcinoma	11 (68.8%)	5 (31.3%)	P=0.671
Squamous cell carcinoma	11 (73.3%)	4 (26.7%)	
Large cell and others	2 (50%)	2 (50%)	
Total	24 (68.6%)	11 (31.4%)	

expression. Henceforth, there was no relation between post-operative tissue samples, XOR expression, and overall survival (Figure 6).

According to the Kaplan Meier analysis performed for overall survival, ECOG PS (p=0.004%) 95% CI: 1.1-4, BRCA1 positivity (p=0.047), and XOR positivity (increased activity) (p=0.05) were included in the multivariable analysis since their *p* values were smaller than 0.2. In multivariable Cox regression, a significant relation was found only between ECOG performance score and overall survival (For ECOG, 2 *vs.* 0, H=10.7, 95% CI: 1.7-65.2, p=0.01). Though the relation between BRCA1 positivity and overall survival rate was not found to be statistically significant, it was close to statistical significance (H=2.6 95% CI: 0.95-7.05, p=0.062).

For XOR, according to the results of Kaplan Meier analysis performed for relapse-free survival ECOG PS (p=0.03) and XOR positivity (p=0.12) were included in the multivariable analysis since their *p* values were smaller than 0.2. In the multivariable Cox regression analysis, no significant relation was found between the two parameters and relapse-free survival. The relation between ECOG performance score and relapse-free survival was only close to statistical significance though it was not significant. (H=4.2, 95% CI: 0.85-20.9, p=0.079) (Table 4).

DISCUSSION

A better understanding of the biology of NSCLC might predict recurrences, further allowing us to increase the quality of life, and to choose the most suit-

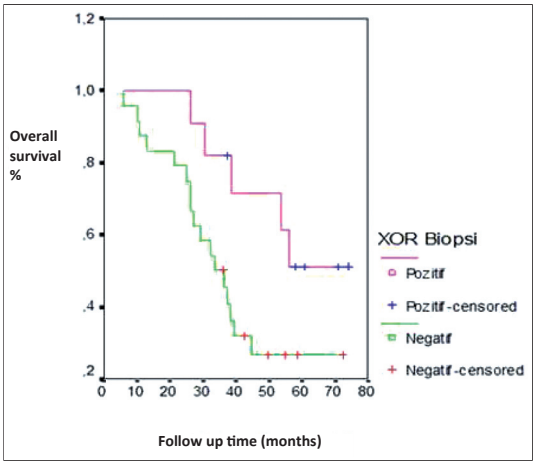

FIGURE 5: Preoperative XOR biopsy and overall survival curve (p=0.0582).
XOR: Xanthine Oxidoreductase.

TABLE 4: Results.

	Total
BRCA1 Biopsy Material (IHC)	
Positive	9 (25.7%)
Negative	26 (74.3%)
BRCA1 Operation Material (IHC)	
Positive	9 (25,7%)
Negative	26 (74,31%)
XOR Biopsy Material (IHC)	
Increased	11 (31.4%)
Decreased	24 (68.6%)
XOR postoperation Material (IHC)	
Increased	9 (25.7%)
Decreased	26 (74.3%)

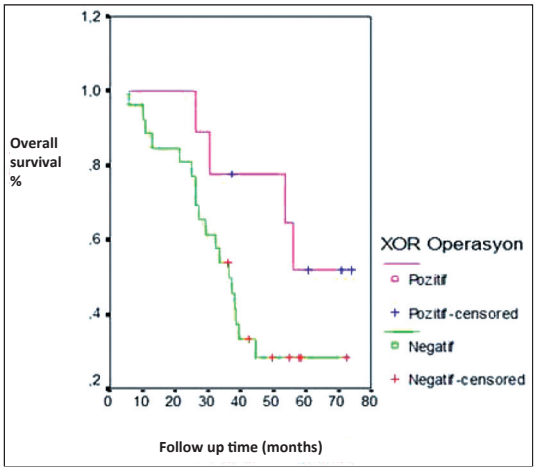

FIGURE 6: XOR postoperation samples and overall survival curve (p=0.109)
XOR: Xanthine Oxidoreductase.

able treatment for survival and recurrences. Previous evidence suggested that the measurement of BRCA1 activity might be beneficial for the treatment strategies of NSCLC.[1-5] The mechanism for the development of platinum resistance and its derivatives is closely associated with BRCA1 activity.[6] Earlier, it was reported that the increase in BRCA expression is an unsatisfactory prognostic factor for lung cancer except for ovarian cancer, as well as, it is a bad predictive factor for cisplatin treatment.[7-9]

The aim of this study was to investigate the prognostic role of BRCA1 and XOR activities especially in patients with locally advanced stage III of NSCLC. We found an increased BRCA1 level in 9 out of 35 patients, with locally advanced NSCLC, during biopsy, and after the operation. The statistical significance between BRCA1 (+) and overall survival was observed as p=0.047. The patients with BRCA1 positive were those who died earlier.

On this basis, we could say that the level of BRCA1 increases, and could be a bad prognostic marker for survival if noted as negative. There was no significance found between BRCA1 expression and the stages. However, BRCA positivity was present in 3 of 8 patients at stage III, and 3 of 10 patients at stage I. Therefore, this study predicted that the higher the stage, the more frequent it is numerically (Table 4).

Previously, it was suggested that chemotherapeutic drugs damage DNA in different ways and lack of BRCA1 function causes increased molecular sensitivity in tumor cells and thus it is a predictive marker for the chemotherapy response in NSCLC.[8] BRCA1 is also involved in nucleotide excision repair like ERCC1. The negative expression of BRCA1 increases cisplatin sensitivity, while its positive expression increases microtubule sensitivity.[5]

However, it is difficult to clearly say that it is a predictive marker, as there were not enough patients in this study. The clinical data suggested that the tumors with BRCA1 value show a low survival rate, and they should be given adjuvant chemotherapy. The prognosis of the patients with BRCA1 was not noted as unsatisfactory as compared to those without BRCA1, and we can infer that, if the number of the patients go up, it might be prognostic for overall survival.

While it was reported in previous studies that chemotherapy causes the tumor tissues of markers, such as ERCC1, to shrink in various degrees.[10] In this study, the subjects were noted as positive for BRCA1 instead of negative. It is difficult to say that chemotherapy or radiotherapy does not have any effect on the IHC expression of this marker since there was a small number of positive BRCA1, just opposite to the data in previous literature.[11]

Xanthine oxidase act as a rate-limiting enzyme in purine metabolism. It has been reported that lack of xanthine oxidoreductase activity is associated with the deterioration of various tumors, and has a bad prognostic factor.[3,9,12-16] The decrease in XOR activity has been observed in a high histologic grade of large tumors and in many breast cancer cases that exhibit high COX–2 expression with bad prognostic features, such as axillary lymph node retention. Also, a decrease in XOR activity was more apparent in the prognosis of the patients with gastric carcinoma.[17-20]

Despite the low survival and deterioration associated with decreased XOR activity, the appropriate cause is not fully understood. Out of 35 biopsy samples, which were negative, 24 belonged to 16 patients (66.6%) who died, and 8 (33.3%) to those who survived. The number of patients with a negative XOR value who died was twice as high as those with a positive value. However, there was no statistical signif-

icance observed between XOR activity and overall survival (p=0.05). Still, according to the data of Kaplan Meier curves, it seems that it might be significant if the number of the patient is higher.

Furthermore, our study investigated that 11 out of 35 patients before the operation, and in 9 out of 35 patients after the operation, showed XOR IHC at an increased level. While 24 patients showed XOR IHC as negative before the operation, and it decreased in 26 patients after the operation. The two patients had increased XOR expression, which further became negative after the operation. On the other hand, XOR expression was found negative in seven out of eight patients at stage III, and five out of ten patients at stage I, while it has been suggested that there is a numeric significance between negativity and progression level (Table 4). There was no statistical significance since the number of patients was low.

No statistical differences were observed between neoadjuvant treatment and the expressions of BRCA1 and XOR. In addition, a partial response was noted from all the patients (29 out of 35).

On the other hand, chemotherapy induces the changes in IHC expression, while a similar effect was not striking for XOR. The XOR activity was noted as negative in two patients. However, these two patients whose XOR activity became negative were the patients whose treatment response was not good. According to COX analysis BRCA1 and ECOG PS, are independent parameters associated with overall survival. Increased BRCA1 expression increases death risk by 2.5 times more than a negative value, and ECOG PS 2 being 2 10 times more. In fact, ECOG PS being 0, is the most effective parameter.

In conclusion, the BRCA1 IHC at tissue level could have a prognostic role in NSCLC though it is not statistically significant from this study. A strong connection could not be made, as there were not enough patients for this study. On the other hand, we are unable to clearly show that lack of XOR activity is a bad prognostic factor in NSCLC, which is mentioned in previous literature. Perhaps, not having enough patients was the reason for it. Therefore, further research is needed by including more number of patients.

Ethics Committee

This study was approved by Baskent University Institutional Review Board Project Number B.30.2.BŞK.0.05.05.05/050.01.08.01-528 Date 07.01.2011.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Selim Yalçın, Ahmet Taner Sümbül, Ali Murat Sedef;* ***Design:*** *Ahmet Taner Sümbül, Ahmet Sezer, Ali Murat Sedef;* ***Control/Supervision:*** *Selim Yalçın, Özgür Özyılkan, Özden Altundağ;* ***Data Collection and/or Processing:*** *Özden Altundağ, Samet Rahatlı;* ***Analysis and/or Interpretation:*** *Ahmet Taner Sümbül, Ahmet Sezer, Samet Rahatlı;* ***Literature Review:*** *Samet Rahatlı, Özden Altundağ;* ***Writing the Article:*** *Ahmet Taner Sümbül, Ali Murat Sedef, Selim Yalçın;* ***Critical Review:*** *Özden Altundağ, Özgür Özyılkan;* ***References and Fundings:*** *Ali Murat Sedef.*

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