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Low-Cost Genetic and Clinical Predictors of Response and Toxicity of Platinum-Based Chemotherapy in Advanced Non-Small Cell Lung Cancer

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Jelena Spasic[1], Milena Cavic[2], Nemanja Stanic[1], Bojan Zaric[3,4], Tomi Kovacevic[3,4], Davorin Radosavljevic[1], and Ljudmila Nagorni-Obradovic[5,6]

Abstract

Background: This study aimed to evaluate for the first time whether certain genetic and clinical factors could serve as minimally invasive predictors of survival and toxicity to platinum-based chemotherapy in advanced lung adenocarcinoma.

Methods: The study included 121 advanced lung adenocarcinoma patients treated with platinum-based dublets until progression or unacceptable toxicity. Response was evaluated using standard radiological methods and toxicity graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Genotyping was performed using PCR-RFLP. Statistical significance was set at $P < .05$.

Results: No significant influence of the examined polymorphisms on the occurrence of high-grade toxicity was detected. However, TP53 72Pro allele carriers were more prone to nausea ($P = .037$) and thrombocytopenia ($P = .051$). Anemia and neuropathy occurred more frequently in XRCC1 399Arg allele carriers (Pearson $\chi 2$ test, $P = .025$ and $P = .004$ respectively). RAD51 135CC carriers were significantly more prone to neutropenia ($P = .027$).

Conclusions: A set of easily determined genetic and clinical predictors of survival and specific toxicity profiles of platinum-based chemotherapy in advanced lung adenocarcinoma were determined in this study, which might be useful for the construction of population-specific, time- and cost-efficient prognostic and predictive algorithms.

Keywords

lung adenocarcinoma, single nucleotide polymorphism, XRCC1, RAD51, TP53

Introduction

According to data from 2020, almost 70% of lung cancer (LC) patients are diagnosed in advanced disease stages when no curative treatment is possible, making it the leading cause of cancer death (18.0% of total).[1] Many demographic, clinical, genetic, and environmental risk factors have been documented[2] contributing to the development of efficient Low-Dose Computed Tomography-based screening programs in individuals with and without smoking history,[3,4] but their significance greatly differs in various populations. The

[1] Clinic for Medical Oncology, Institute for Oncology and Radiology of Serbia, Serbia
[2] Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Serbia
[3] Faculty of Medicine, University of Novi Sad, Serbia
[4] Institute for Pulmonary Diseases of Vojvodina, Serbia
[5] Faculty of Medicine, University of Belgrade, Serbia
[6] Clinic for Pulmonology, University Clinical Centre of Serbia, Serbia

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Corresponding Author:
Jelena Spasic, MD, Clinic for Medical Oncology, Institute for Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia.
Email: jelena.spasic@ncrc.ac.rs

epidemiological situation in Serbia is similar to the global one.[5] Lung cancer is the most frequently diagnosed cancer in men (21.2% of all malignant tumors), with standardized incidence and mortality rates of 57, that is, 53.3 per 100.000, respectively. In women, it is the second most frequent malignant localization after breast cancer (10.2% of all malignant tumors) with standardized incidence and mortality rates of 22.4, that is, 19.8 per 100.000, respectively. Although targeted molecular characterization has redefined treatment strategies for non-small cell lung cancer (NSCLC), platinum-based chemotherapy still has a place in the treatment of advanced NSCLC without actionable oncogenic drivers or with contraindications for immunotherapy.[6,7] Platinum doublets consisting of cisplatin or carboplatin with one of the third-generation cytotoxic drugs (paclitaxel, gemcitabine, docetaxel, vinorelbine) have shown comparable efficacy and toxicity profiles.[8,9]

Our group and others have made investigator-initiated efforts to explore genetic and clinical biomarkers for LC in Serbia, in order to enravel new risk factors and ensure closer monitoring of LC patients using available cost-efficient methods.[10-13] Some of the genetic factors had proven significant for the development of LC in our population, but also might affect the way the cell responds to and processes platinum-based chemotherapy, which depends in part on DNA repair mechanism. X-ray cross complementing group 1 protein (XRCC1) participates in the assembly of DNA single-strand-break repair complex, but is also involved in other putative repair axes, which makes this protein essential for cellular defense against damage.[14] XRCC1 single nucleotide polymorphisms (SNPs) might influence the development of different cancer subtypes and interfere with the response to anti-cancer treatment, but the data is still inconclusive.[15,16] RAD51 is a component of the homologous recombination and double-stranded-break DNA repair machinery, thus directly affecting the cell's ability to respond to DNA damage induced by chemotherapy.[17] RAD51 is a highly polymorphic molecule and various SNPs have been shown to affect its expression and function ultimately modulating the cell's DNA repair efficiency.[18,19] Under physiological conditions, activation of TP53 induces apoptosis, cell cycle arrest or cell senescence and protects the tissue from damage, but in situations when TP53 is not functioning correctly due to aberrant expression and/or genetic changes, cells are not adequately protected.[20] Various TP53 SNPs have been studied in this context, as they alter its function and underlying biochemical pathways.[21]

A rise in the number of patients diagnosed with advanced LC is expected in the next period, due to a temporary stop/slowing down of LC screening programs and diagnostic procedures during the COVID-19 pandemic.[22,23] As targeted treatment options and immunotherapy for advanced LC are limited by the detection of predictive biomarkers, chemotherapy still has an important role in patient treatment strategies, especially in low- and middle-income countries due to inaccessibility of drugs in various indications. Very limited literature data is available on genetic predisposition to specific types of toxicity combining clinical and genetic factors in NSCLC patients treated with platinum-based chemotherapy. Thus, the aim of this research was to evaluate for the first time whether some easily determined clinical and genetic factors, as SNP might serve as low-cost and minimally invasive predictors of response and toxicity of platinum-based chemotherapy in advanced lung adenocarcinoma, which could be useful for the construction of population-specific, time- and cost-efficient prognostic and predictive algorithms.

Materials and methods

Patients and Treatment

This study included 121 patients with histologically or cytologically confirmed primary advanced (stage IIIB,C and IV) lung adenocarcinoma according to the eighth WHO classification,[24] older than 18 years, evaluated in the period from 2015 to 2018 with available complete medical records and clinical follow up. Epidermal growth factor receptor (EGFR) gene mutation testing on exons 18–21 was performed using the Cobas® EGFR Mutation Test v2 on Cobas® 4800 (Roche Diagnostics).[25,26] Other molecular testing was not part of routine practice in Serbia at the time this study was conducted. Patients without EGFR mutations were treated with platinum-based dublets in the first line according to national treatment guidelines and reimbursement at the time, in the period between 2015 and 2018, until progression or unacceptable toxicity. Response to therapy was evaluated by standard radiological methods according to Response evaluation criteria in solid tumors (RECIST) 1.1. Objective response rate (ORR) was defined as the percentage of patients who achieved a partial (PR) or complete response (CR) to therapy, and disease control rate (DCR) as the percentage of patients who derive any benefit form therapy (PR+CR+stable disease (SD)). Progression-free survival (PFS) was defined as the time from the start of chemotherapy to date of progression and overall survival (OS) as the time from diagnosis to death from any cause. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.[27] Patients were followed by clinic visits or telephone until death. All patients signed an informed consent for participation in the study. The procedures used in this study were approved by the Ethics Board of the Institute for Oncology and Radiology of Serbia and were in accordance with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards.

In silico analysis of TP53, XRCC1, and RAD51 expression and interactions using the Human Protein Atlas and STRING databases

The interactive open-access databases the Human Protein Atlas (HPA)[28] was used to analyze the publicly available Cancer Genome Atlas (TCGA)[29] transcriptome data on TP53, XRCC1, and RAD51. Kaplan–Meier curves present a summary of correlation analysis between mRNA expression levels

and patient survival, by dividing patients into low (under experimental cut-off) or high (above experimental cut-off) expression groups. Corresponding images and data were downloaded from the HPA platform in the original form. The STRING[30,31] analysis network of TP53, XRCC1, and RAD51 was built based on high confidence (.7) evidence from experimental interaction data, co-expression data, gene fusions, gene co-occurrence, gene neighborhood, predictive and knowledge text mining and curated metabolic and signaling pathway databases imported from KEGG. The analysis was extended to include primary-interaction shell genes and clusters to explore indirect interactions of TP53, XRCC1, and RAD51 and the possible effects of these interactions. The analysis was performed using STRING v.11.0[30] and corresponding images and data downloaded in the original form with statistical significance set at $P < .05$.

TP53, XRCC1, and RAD51 Genotyping

The analysis was conducted on 121 patients, which meets the criteria of a representative minimal number of necessary samples of NSCLC in Serbia according to its incidence and population size using the 95% confidence level.[32,33] TP53 Arg72Pro (HGNC[34] ID:11998, rs1042522[35]), XRCC1 Arg399Gln (HGNC[34] ID:12828, rs25487[35]), and RAD51 G135C (HGNC[34] ID:9817, rs1801320[35]) polymorphisms were analyzed from FFPE derived DNA using a standard PCR-RFLP approach.[11,36] Briefly, the TP53 Arg72Pro SNP was detected using the following primers: sense: 5′-ATCTA-CAGTCCCCCTTGCCG-3′ and antisense: 5′-GCAACT-GACCGTGCAAGTCA-3′ resulting in a 296 bp PCR product. Thermal cycling conditions were as follows: initial denaturation step at 95°C for 3 min, 35 cycles of: 95°C 30 s, 58°C 30 s, 72°C 40 s and the final elongation for 10 min at 72°C. PCR products were digested with Bsh1236I (Thermo Scientific) fast digest restriction enzyme. XRCC1 Arg399Gln polymorphism was determined using the following primers: sense: 5′-CAAGTA-CAGCCAGGTCCTAG-3′, antisense: 5′-CCTTCCCTCATCTGGAGTAC-3′ resulting in a 268bp PCR product. Thermal cycling conditions were as follows: initial denaturation step at 94°C for 5 min, 32 cycles of: 94°C 60 s, 58°C 30 s, 72°C 40 s and the final elongation for 10 min at 72°C. PCR products digested with BcnI (Thermo Scientific) fast digest restriction enzyme. The RAD51 G135C polymorphism was determined using the following primers: sense: 5′-TGGGAACTGCAACTCATCTGG-3′, antisense: 5′-GCGCTCCTCTCTCCAGCAG-3′ resulting in a 157 bp PCR product. Thermal cycling conditions were as follows: initial denaturation step at 94°C for 3 min, 35 cycles of: 94°C 60 s, 54°C 30 s, 72°C 40 s and the final elongation for 7 min at 72°C. Obtained PCR products were digested with MvaI (Thermo scientific) fast digest restriction. PCR products and corresponding restriction digestion fragments were analyzed on the Agilent 2100 Bioanalyzer. A previously sequenced heterozygote sample was used for each polymorphism as a control to assure adequate genotyping and the genotyping was performed blind to case-control status. Randomly selected 10% of samples were analyzed by Sanger sequencing to ensure data validity.

Statistical Analyses

Descriptive methods of statistical analysis (frequencies, percentage, mean, median, standard deviation/SD/and range) were used to summarize the sample data. The associations between the patients' and tumor characteristics were analyzed using Pearson chi-square with Bonferroni correction or Fisher's exact t-test, Wilcoxon–Mann–Whitney or Kruskal–Wallis test. Logistic regression was used to calculate the odds ratio (OR) and the 95% confidence interval (CI) to identify factors significant for outcome, and for testing of the significance of individual factors in the model the Likelihood Ratio and Wald test were used. Survival analysis was performed using a standard Kaplan–Meier product-limit method for graphical presentation; median with corresponding 95% confidence interval (95%CI) was used for description and the log-rank test for the analysis of difference. Cox regression analysis was performed to obtain the hazard ratios (HR) when survival between analyzed groups was significant. Descriptive analyses included genotype and allelic frequencies, and their distribution between groups was tested by Fisher's exact test. Two-sided P values <.05 were considered to indicate statistical significance. The analyses were performed using SPSS Statistics 21 (IBM-SPSS program ver. 21) and Rcmdr (R Commander, V2.6-1, GLP).

Results

Patient Treatment and Response

Patient characteristics are presented in Table 1. All patients received at least 2 cycles of platinum-based chemotherapy, and the median number of cycles was 4 (1–6). Most patients received cisplatin, 101 (83.5%), while only 20 patients (16.5%) received carboplatin in combination with a third-generation cytotoxic drug (Supplementary Table 1) Objective response rate (ORR) was 21% and disease control rate (DCR) 77%. Median PFS was 5.6 months (95% CI 4.8–6.5), and median OS 10.0 months (95% CI 8.1–12.0). Toxicity of any grade was reported in 62 patients (51%), and included leucopenia in 37 patients (30.6%), neutropenia in 40 (33%), thrombocytopenia in 21 (17.4%), anemia in 43 (35.5%), diarrhea in 13 (10.7%), nausea 31 (25.6%), emesis in 8 (6.6%), neuropathy in 12 (9.9%), elevated creatinine level in 14 (11.6%) and elevated transaminase levels in 1 patient (.8%). High-grade toxicity, which was defined here as toxicity grade 3 or higher, was reported in 46 (38%) patients, mainly hematologic, in 43 patients. Two patients died as a result of neutropenic sepsis. At the time of this analysis, 98.3% of patients had died.

No significant difference in mPFS and mOS was detected in relation to the type of chemotherapy protocol, type of

Table 1. Patient characteristics.

Characteristics	Patients N (%)
Gender	
Male	79 (65)
Female	42 (35)
Age (years)	
Range	37–84
Median	61
Smoking status	
Non-smoker or ex-smoker ≥30 years	22 (18)
Smoker or ex-smoker <30 years	99 (82)
ECOG PS[a]	
0	3 (2.5)
1	114 (94.2)
2	3 (2.5)
NA[b]	1 (.8)
Presence of metastasis at diagnosis	
No	28 (23)
Yes	91 (75)
NA[b]	2 (2)
Tumor (T)	
T1	10 (8.3)
T2	35 (28.9)
T3	28 (23.1)
T4	45 (37.2)
Tx	3 (2.5)
Node (N)	
N0	19 (15.7)
N1	13 (10.7)
N2	31 (25.6)
N3	52 (43.0)
Nx	6 (5.0)
Metastasis (M)	
M0	3 (2.5)
M1	114 (94.2)
M2	4 (3.3)

[a]ECOG PS—The Eastern Cooperative Oncology Group Performance Status.
[b]NA—data unavailable.

platinum derivative, demographic characteristics, smoking status, T, N, M, or disease stage. However, ECOG performance status was found to be a prognostic factor of survival, as patients with ECOG PS2 had significantly shorter mPFS [ECOG PS0 vs PS1 vs PS2, 10.02 (9.28–10.76) vs 5.55 (4.73–6.37) vs 2.63 (3.91–4.31) months, P = .04.] (Figure 2a).

In silico expression analysis of TP53, XRCC1, and RAD51 using the Human protein Atlas and prediction of their interactions using STRING

In silico expression analysis showed that TP53, XRCC1, and RAD51 were prognostically significant in lung cancer (Supplementary Figure 1a-c). Survival analysis of publicly available TCGA data showed that high expression of TP53 is a favorable prognostic factor, with a 5-year survival rate of 47% in the high expression group and 38% in the low expression group (cut-off 10.76y, P = .050, Supplementary Figure 1a). High expression of XRCC1 was also correlated with longer survival, with a 5-year survival rate of 48% in the high expression group and 39% in the low expression group (cut-off 9.96y, P = .051, Supplementary Figure 1b). In the case of RAD51, low expression was found to be prognostically significant (cut-off 1.48y, P = .003, Supplementary Figure 1c).

The simple STRING analysis and showed that TP53 has significant direct interactions in the cell with XRCC1 and RAD51 (co-expression, experimental/biochemical data; PPI enrichment *P* value = .045) at a high confidence setting (.7) (Supplementary Figure 1d). After extending the network to 5 primary-interaction shell genes (MND1, ATM, MDM2, EP300, CREBBP) to explore further indirect interactions between TP53, XRCC1, and RAD51, the analysis showed an enrichment in interactions using the intersection of 8 molecules present on all analyzed platforms (PPI enrichment *P* value = .026) (Supplementary Figure 1e). This result implied that TP53, XRCC1, and RAD51 are biologically interconnected with each other, more than is expected for randomly selected molecules of similar size. The additional cluster analysis confirmed that the connection between XRCC1 and RAD51 is achieved through the TP53 axis (Supplementary Figure 1f).

Other than the biological connection of these 3 genes, their 3 SNPs, TP53 Arg72Pro, XRCC1 Arg399Gln, and RAD51 G135C were previously correlated with lung cancer risk in our population.[11] Thus, they were further evaluated in this study in relation to response to platinum-based chemotherapy.

TP53, XRCC1, and RAD51 Genotyping and Correlation with Survival

A 296 bp PCR product containing the TP53 Arg72Pro polymorphic site was obtained from 106 patient samples (Figure 1a). The distribution of the genotypes did not deviate from the Hardy–Weinberg equilibrium ($\chi 2 = .0002$; P = .988). Allele frequencies were .57 for Arg and .43 for Pro. A 268 bp PCR product containing the XRCC1 Arg399Gln polymorphic site was obtained from 103 patient samples (Figure 1b). The distribution of the genotypes (Table 2) did not deviate from the Hardy–Weinberg equilibrium ($\chi 2 = .3715$; P = .542). Allele frequencies were .75 for Arg and .25 for Gln. A 157 bp PCR product containing the RAD51 G135C polymorphic site was obtained from 109 patient samples (Figure 1c). The distribution of the genotypes (Table 2) did not deviate from the Hardy–Weinberg equilibrium ($\chi 2 = .2246$; P = .636). Allele frequencies were .84 for G and .16 for C. There were no significant differences in genotype distributions of all 3 analyzed polymorphisms according to gender, smoking status, ECOG PS or other clinical factors.

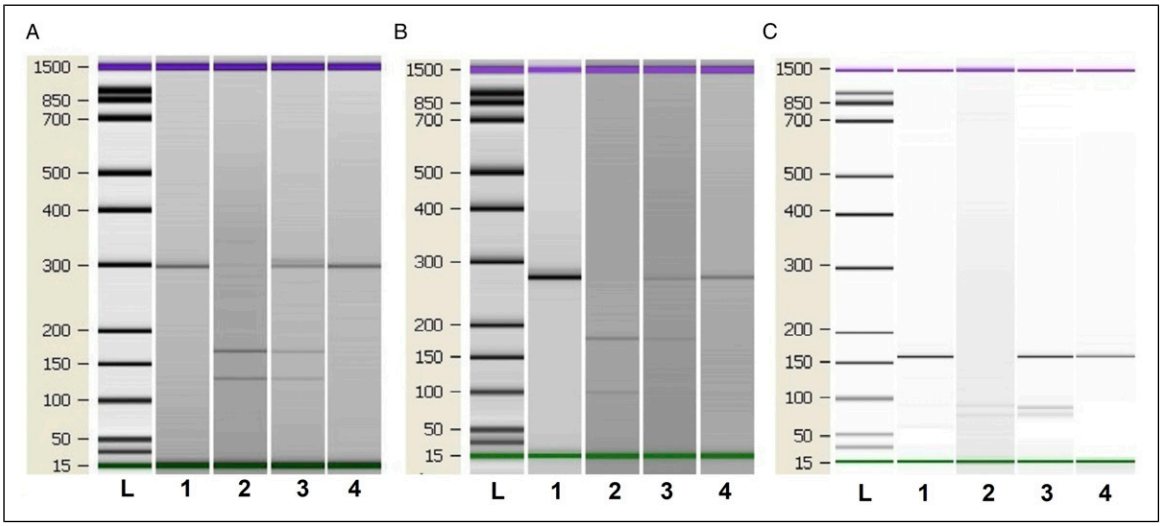

Figure 1. a) PCR and RFLP results of TP53 Arg72Pro polymorphic variants. Column 1: 296 bp PCR product. Column 2: Arg/Arg, Column 3: Arg/Pro, Column 4: Pro/Pro. b) PCR and RFLP results of XRCC1 Arg399Gln polymorphic variants. Column 1: 268 bp PCR product. Column 2: Arg/Arg, Column 3: Arg/Gln, Column 4: Gln/Gln. c) PCR and RFLP results of RAD51 G135C polymorphic variants. Column 1: 157 bp PCR product, Column 2: G/G, Column 3: G/C, Column 4: C/C. L–High-sensitivity DNA ladder. 1500 bp upper and 15 bp lower marker are present in each column.

Table 2. The effects of TP53 Arg72Pro, XRCC1 Arg399Gln, and RAD51 G135C polymorphic variants on toxicity using dominant and recessive models.

Gene/model	Genotype	Patients with toxicity vs without OR (95% CI)	*P* value (Pearson χ^2 Test)
TP53 Arg72Pro			
dominant model		.51 (.22–1.17)	.082[a]
recessive model	ArgArg vs any Pro any Arg vs ProPro	1.61 (.60–4.33)	.457
XRCC1			
Arg399Gln	ArgArg vs any Gln any Arg vs GlnGln	.83 (.38–1.82)	.691
dominant model		1.40 (.32–6.22)	.653
recessive model			
RAD51 G135C			
dominant model	GG vs any C	.86 (.38–1.96)	.835
recessive model	Any G vs CC	1.02 (.06–16.71)	1.000
Combinations			
TP53/RAD51	ArgArg/GG vs ArgArg/GC+CC	.07 (.01–.70)	**.03**
TP53/XRCC1	ArgArg/ArgArg vs ArgArg/ArgGln+GlnGln	1.27 (.28–5.87)	.76
XRCC1/RAD51	ArgArg/GG vs ArgArg/GC+CC	2.22 (.57–8.65)	.40
	ArgArg+ArgGln/GG vs ArgArrg+Gln/GC+CC	1.33 (.49–3.60)	.75

Statistically significant results are labeled bold.
[a]statistical trend.

The effects of the polymorphic variants were explored employing dominant and recessive models. Employing the dominant model for the TP53 Arg72Pro polymorphism (ArgArg vs ArgPro+ProPro), a significant effect on PFS [5.65 months (3.74–7.57) vs 6.01 months (4.71–7.31), Log Rank (Mantel–Cox) P = .831], and OS was not detected [13.2 months (10.0–16.5) vs 10.7 months (8.1–13.3), Log Rank (Mantel–Cox) P = .288]. When the recessive model was used (ArgArg+ArgPro vs ProPro), no significant effect was detected on PFS [5.9 months (4.9–7.0) vs 5.9 months (.0–12.4), Log Rank (Mantel–Cox) P = .909] or OS [12.1 months (9.3–14.9) vs 11.0 months (6.3–15.7), Log Rank (Mantel–Cox) P = .754].

Using the dominant model for the XRCC1 Arg399Gln polymorphism (ArgArg vs ArgGln+GlnGln), ArgArg carriers were found to have longer OS [median 15.0 months (13.1–16.9) vs 8.6 months (6.2–10.9), Log Rank (Mantel–Cox) P = .121; Breslow (Generalized Wilcoxon) P = .009] (Figure 2),

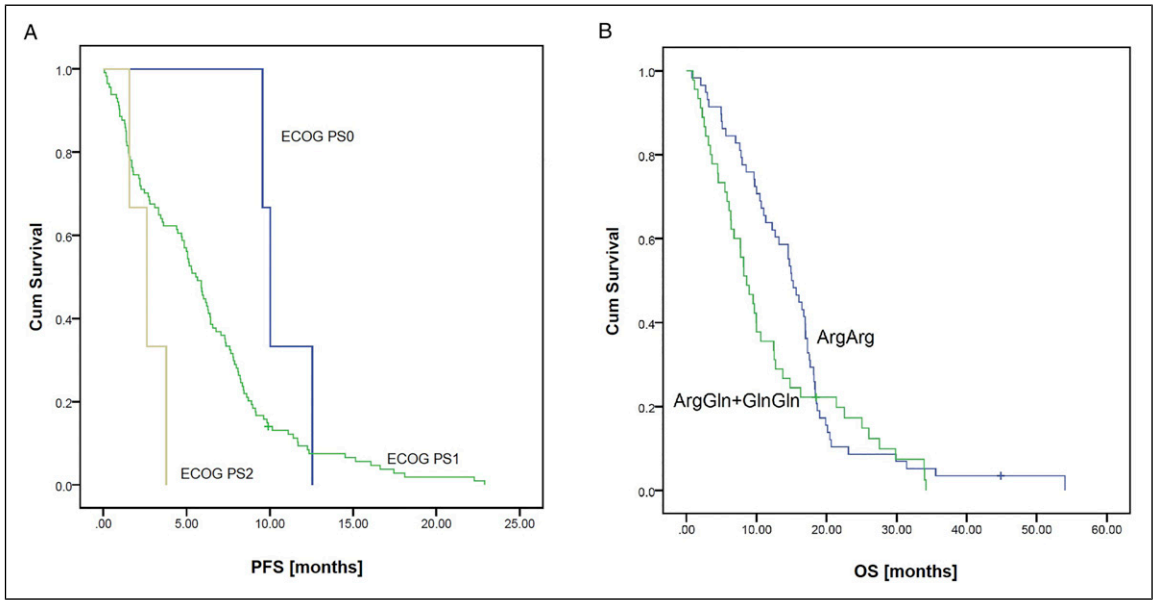

Figure 2. a) Median progression-free survival (mPFS) according to ECOG performance status. b) Median overall survival (mOS) according to the distribution of XRCC1 Arg399Gln polymorphic variants applying the dominant model.

while statistical significance was not confirmed for PFS [6.1 months (4.6–7.7) vs 5.26 months (2.5–7.8), Log Rank (Mantel–Cox) P = .397]. When the recessive model was used (ArgArg+ArgGln vs GlnGln), no significant effect was confirmed on PFS [5.9 months (4.8–6.9) vs 5.3 months (2.7–8.0), Log Rank (Mantel–Cox) P = .262] or OS [12.6 months (9.2–16.1) vs 8.6 months (6.1–11.0), Log Rank (Mantel–Cox) P = .158].

Applying the dominant model for the RAD51 G135C polymorphism (GG vs GC+CC) no significant effect was confirmed on PFS [5.9 months (4.7–7.1) vs 5.1 months (2.1–8.0), Log Rank (Mantel–Cox) P = .941] or OS [11.0 months (8.1–13.9) vs 12.1 months (7.9–16.2), Log Rank (Mantel–Cox) P = .242]. When the recessive model was used (GG+GC vs CC), no significant effect was confirmed on PFS [5.6 months (4.8–6.5) vs 7.8 months, Log Rank (Mantel–Cox) P = .503] or OS [11.0 months (8.5–13.4) vs 12.1 months, Log Rank (Mantel–Cox) P = .927].

Correlation of TP53, XRCC1, and RAD51 Polymorphisms with Toxicity

We found no significant influence of any of the examined polymorphisms on the occurrence of toxicity in general, or of high-grade toxicity of platinum-based chemotherapy, using either the dominant or recessive models of association (Table 2). However, some specific significant results were obtained.

Employing the dominant model for the TP53 Arg72Pro polymorphism (ArgArg vs ArgPro + ProPro), a trend was observed for a more frequent occurrence of toxicity in Pro allele carriers, but statistical significance was not reached (Pearson $\chi 2$ test, P = .082). Pro allele carriers were found to be more prone to developing nausea (Pearson $\chi 2$ test, P = .037) and thrombocytopenia of any grade (P = .049, Pearson $\chi 2$ test). When the recessive model was used (ArgArg +ArgPro vs ProPro), ProPro homozygote carriers were found to be significantly more prone to thrombocytopenia (Pearson $\chi 2$ test, P = .045), especially high-grade (Pearson $\chi 2$ test, P = .014). Employing the recessive model for the XRCC1 Arg399Gln polymorphism, Arg allele carriers were found to be significantly more prone to developing anemia (Pearson $\chi 2$ test, P = .025) and neuropathy (Pearson $\chi 2$ test, P = .004). Using the recessive model for the RAD51 G135C polymorphism (GG vs GC+CC) it was found that neutropenia occurred significantly more frequently in CC homozygote carriers (P = .027, Pearson $\chi 2$ test). Taking into account possible epistatic interactions, of all the tested polymorphic variants combinations (excluding the TP53 Pro allele which carries an independent risk for toxicity), it was found that carriers of the TP53/Rad51 ArgArg/GC + CC genotypes were significantly more prone to developing toxicity in general (P = .030, Pearson $\chi 2$ test).

Discussion

In order to profile new biomarkers of specific toxicity and survival, we aimed to evaluate the significance of clinical factors and genetic polymorphisms of TP53 and DNA repair enzymes XRCC1 and RAD51 that were previously shown to be potential risk factors for NSCLC and other types of cancer in our population.[11,36] Some of these polymorphisms have been evaluated in connection with response to platinum-based chemotherapy in NSCLC patients, mostly in Asian populations, but the data is still inconclusive and depends on various population-specific parameters.[37-39] The importance

of developing low-cost prognostic tools capable of predicting toxicity to anti-cancer therapy is highlighted by the high cost of large predictive NGS panels and whole genome analysis which impairs their use in everyday clinical practice, especially in developing countries. Performing simple pharmacogenomic background profile check of patients might be useful for predicting toxicity occurrence and point to patients which need to be monitored more closely to increase their quality of life.

The response patterns to first-line chemotherapy and the median PFS and OS were in accordance with literature data for advanced NSCLC.[6] The reported toxicity included hematological (leucopenia, neutropenia, thrombocytopenia, anemia), diarrhea, nausea, emesis, neuropathy and elevated levels of creatinine and transaminases, all characteristic for platinum-based chemotherapy. Of all analyzed factors, worse ECOG performance status (PS2) correlated with significantly shorter progression-free survival. This result is in accordance with literature data on chemotherapy, as well as emerging data on immunotherapy,[40] for which it represents a useful prognostic and predictive factor.

A significant effect of the 3 tested polymorphic variants individually on PFS and OS was not confirmed in this study group. However, it was detected that TP53 Arg72Pro Arg carriers had a trend of longer PFS and OS and that chemotherapy dose reduction was significantly more frequently necessary in Pro allele carriers. Also, Pro allele carriers exhibited toxicity in general more frequently, as well as nausea and thrombocytopenia specifically, especially in the ProPro homozygous state. XRCC1 Arg399Gln ArgArg carriers were found to have longer PFS and OS although statistical significance was not reached, probably due to low sample size. Arg allele carriers were more prone to exhibit anemia and neuropathy. RAD51 G135C CC recessive homozygote carriers more often developed neutropenia. When testing all 3 polymorphic variants combinations (excluding the TP53 Pro allele which carries an independent risk for toxicity), it was found that carriers of the TP53/Rad51 ArgArg/GC+CC genotypes were significantly more prone to exhibiting toxicity.

The functional TP53 SNP Arg72Pro at codon 72 in exon 4 introduces a replacement of the positively charged arginine amino acid with proline in the corresponding TP53 protein, leading to a conformational change and its lower ability to induce apoptosis. On the other hand, the Pro allele is considered to be more prone to inducing G1-cell cycle arrest activating DNA repair.[41] Thus, the observed prolonged survival of carriers of the wild type Arg allele and toxicity observed in Pro allele carriers might be explained by changes in the conformation of TP53 which led to enhanced DNA repair and rescue of cancer cells inducing resistance to chemotherapy. The G>A substitution at position 28152 in exon 10 of XRCC1 leads to a change from arginine to glutamine in the XRCC1 protein, inducing significant conformational alterations in the region crucial for protein–protein interactions within the DNA repair scaffolding machinery.[42,43] The functional RAD51 SNP G135C represents a nucleotide change in codon 150 of the 5′ untranslatable gene region, which is considered to affect the transcription of RAD51 as it is located in a CpG island.[44] Thus, the activity of these 2 DNA repair proteins should also be evaluated prior to chemotherapy as the presence of these common SNPs can affect the sensitivity of tumor cells, as well as normal cells, and lead to the appearance of toxicity or changes in survival rates.[16] The results of this study suggest that performing a simple pharmacogenomic background profile of patients might be useful for predicting toxicity occurrence and point to patients which need to be monitored more closely during platinum-based chemotherapy to increase their quality of life.

This study is limited by its retrospective nature, and the fact that it has been performed on a relatively small number of patients from 2 oncological centers in Serbia. However, to the best of our knowledge, this is the first study performed on advanced NSCLC patients treated with platinum-based chemotherapy that analyzed genetic predisposition to specific types of toxicity combining clinical and genetic factors. Multicenter studies performed on patients from different populations are warranted to evaluate the potential of these results for the construction of population-specific, time- and cost-efficient prognostic algorithms for chemotherapy-treated NSCLC patients in the future.

Conclusions

A set of easily determined and genetic and clinical predictors of survival and specific toxicity profiles to platinum-based chemotherapy in advanced non-small cell lung cancer were determined in this study, which might be useful for the construction of population-specific, time- and cost-efficient prognostic and predictive algorithms in this setting.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics statement

The procedures used in this study were approved by the Ethics Board of the Institute for Oncology and Radiology of Serbia and were in accordance with the Helsinki Declaration of 1964 and its later

amendments or comparable ethical standards. All patients signed an informed consent.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to ethics restrictions (their containing information could compromise the privacy of patients).

ORCID iDs

Jelena Spasic https://orcid.org/0000-0002-1386-9114
Milena Cavic https://orcid.org/0000-0002-7604-9295
Nemanja Stanic https://orcid.org/0000-0002-6840-148X

Supplemental Material

Supplemental material for this article is available online.

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