

Chemosensitivity and survival of non-small cell lung carcinoma patients receiving neoadjuvant therapy depend on the expression of multidrug efflux transporters

Tijana Stankovic^{1,*}, Jelena Stojic², Miodrag Dragoj¹, Zorka Milovanovic³, Zorica Milosevic¹, Vedrana Milinkovic¹, Vesna Skodric-Trifunovic^{4,5}, Ljiljana Denic-Markovic⁶, Milica Pesic¹, Sonja Stojkovic Buric¹, Nikola Tanic¹ and Jasna Bankovic¹

¹Department of Neurobiology, Institute for Biological Research "Sinisa Stankovic" - National Institute of Republic of Serbia, University of Belgrade, 11060 Belgrade, Serbia

²Department of Thoracopulmonary Pathology, Service of Pathology, Clinical Centre of Serbia, 11000 Belgrade, Serbia

³Institute for Oncology and Radiology of Serbia, University of Belgrade, 11000 Belgrade, Serbia

⁴Clinic for Pulmonology, Clinical Center of Serbia, 11000 Belgrade, Serbia

⁵Faculty of Medicine, University of Belgrade, 11000 Belgrade, Serbia

⁶Institute for Epidemiology, Faculty of Medicine, University of Belgrade, 11000 Belgrade, Serbia

*Correspondence: tijana.andjelkovic@ibiss.bg.ac.rs (Tijana Stankovic)

DOI: [10.31083/j.jmcm.2019.04.3241](https://doi.org/10.31083/j.jmcm.2019.04.3241)

This is an open access article under the CC BY-NC 4.0 license (<https://creativecommons.org/licenses/by-nc/4.0/>).

The current study aimed to evaluate the impact of neoadjuvant chemotherapy (NACT) on the expression of three major multidrug resistance efflux transporters, P-glycoprotein (P-gp/ABCB1), multidrug resistance associated protein (MRP1/ABCC1) and breast cancer resistance protein (BCRP/ABCG2) in non-small cell lung carcinoma (NSCLC) patients. Particularly, their role as molecular markers of chemosensitivity and prognosis of NSCLC patients receiving NACT was investigated. To that end, we specifically studied mRNA and protein expression of these three efflux transporters in two independent groups, each consisting of 35 NSCLC patients who did or did not receive platinum-based NACT. Alterations in the expression of MDR efflux transporters were statistically analyzed in relation to NACT status, and their associations were evaluated regarding patients' survival. The frequency of samples with positive MRP1 expression was significantly decreased in the NACT group, regardless of the applied platinum drugs which are known to induce MRP1 expression. On the other hand, the incidence of BCRP expressing tumor specimens, doubly positive BCRP and P-gp as well as triple positive samples increased in the NACT group. Importantly, patients lacking P-gp expression had more favorable prognosis with NACT than without NACT, whereas the status of MRP1 and BCRP did not influence the patients' survival in both investigated groups. Collectively, we show that decreased MRP1 and increased BCRP expression after NACT could determine the chemosensitivity of NSCLC following adjuvant therapy, whereas P-gp expression status could be considered a prognostic marker for NSCLC patients who can benefit from NACT treatment.

Keywords

P-gp; ABCB1; MRP1; ABCC1; BCRP; ABCG2; neoadjuvant chemotherapy; non-small cell lung carcinoma

1. Introduction

Non-small cell lung carcinoma (NSCLC) is the most common histological type of lung carcinoma, accounting for 85% of all lung cancer cases [1]. Platinum-based chemotherapy is the treatment of choice for most NSCLC stages [2]. When combined with surgery, chemotherapy is usually applied after tumor resection (adjuvant chemotherapy). However, for NSCLC patients with good performance status, neoadjuvant chemotherapy (NACT), prior to surgery, is also considered a treatment strategy [3]. Despite chemotherapy administration, NSCLC patient survival remains unsatisfactory with median relative survival less than 1 year [4]. One reason for the dismal prognosis of NSCLC is the poor response to chemotherapy due to the frequent development of chemoresistance.

The most extensively studied mechanism of drug resistance is increased drug efflux and reduced intracellular drug concentration due to the overexpression of ATP-binding cassette (ABC) multidrug efflux transporters. This transporter superfamily has several subclasses including P-glycoprotein/multidrug resistance protein (P-gp, MDR1, ABCB1), multidrug resistance associated protein (MRP1, ABCC1) and breast cancer resistance protein (BCRP, ABCG2). These ATP-driven pumps extrude a variety of xenobiotics out of cells, including the majority of chemotherapeutics [5]. Under physiological conditions, these transporters are widely expressed in human lung tissues, with an important role in protection against cytotoxins. However, mutations, polymorphisms and altered expression of ABC transporters may cause develop-

ment of lung diseases, predominantly cancer [6]. Preclinical studies suggest that ABC transporters are involved in the development of resistance to chemotherapeutics in NSCLC. Major MDR efflux pumps were shown to confer chemoresistance *in vitro* and *in vivo* to multiple unrelated drugs, including platinum agents, key components of NSCLC chemotherapy protocols [7, 8, 9, 10, 11]. However, results from clinical studies are inconsistent regarding the association of ABC transporters with chemotherapy response and prognosis in NSCLC. For example, Li and colleagues reported a significant association between high MRP1 expression and poor response to cisplatin-based chemotherapy and survival [12], whereas in another, larger study no significant associations were found [13]. Similar, conflicting results were also reported for BCRP expression and response rate to platinum-based therapy [12, 14]. However, all these studies considered associations between ABC transporters and adjuvant chemotherapy response, with only a few studies investigating associations of these efflux pumps with the effect of NACT. Therefore, in this study, we examined the potential role of P-gp, MRP1 and BCRP, as molecular markers of chemosensitivity associated with NACT treatment in NSCLC. We performed a comprehensive analysis of their mRNA and protein expression levels in two independent groups of 35 NSCLC patients who did or did not receive platinum-based NACT. We further investigated the association between ABC pump expression and NACT status. Finally, we analyzed their effects on patients' survival to study the potential prognostic value of P-gp, MRP1 and BCRP for NSCLC patients receiving NACT.

2. Materials and methods

2.1 Tissue samples

Two groups of NSCLC samples were collected and analyzed in this study. One group included 35 patients who received one to four cycles of neoadjuvant platinum-based chemotherapy (**NACT group**), while the other group of 35 patients did not receive NACT (**non-NACT group**). All 70 patients underwent surgery between June 2004 and September 2011. Lobectomy or pneumonectomy with lymphadenectomy was conducted at the Clinic of Thoracic Surgery, Clinical Centre of Serbia and pathological diagnosis was performed at the Department of Thoracopulmonary pathology, Service of Pathology, Clinical Centre of Serbia. Tumor and surrounding non-tumor lung tissues from each patient were obtained for diagnosis and investigation. Surrounding non-tumor tissue samples were taken at a distance of at least 3 cm away from the primary tumor lesion. The samples were fixed in 10% buffered formalin, embedded in paraffin for diagnostic procedures and immunohistochemical analyses. For mRNA expression analysis of NACT samples, RNA was extracted from paraffin blocks. For the non-NACT group, patient's tissues were freshly frozen in liquid nitrogen and kept until RNA extraction. Diagnosis of NSCLC and histopathological classification were established by histological examinations in our previous publication [15]. All samples were collected and used after obtaining patients' informed consent and approval from the Ethics Committee of the Faculty of Medicine, University of Belgrade, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2 RNA isolation and Reverse Transcription

Total RNA was isolated from pairs of tumor tissues and corresponding non-tumor tissues of all NSCLC patients. The RNA from paraffin blocks was extracted using RecoverAll™ Total Nucleic Acid Isolation kit (Ambion, USA), while RNA isolation from fresh frozen samples was carried out using Trizol® reagent (Invitrogen Life Technologies, USA). Both procedures were performed according to the manufacturers' protocols. Isolated RNA was quantified spectrophotometrically and the quality was verified by agarose gel electrophoresis. Reverse transcription reactions were performed using 2 µg of total RNA, with a High-capacity cDNA reverse transcription kit (Applied Biosystems, USA), following the manufacturer's protocol.

2.3 Quantitative real-time PCR

Quantitative real time PCR (qRT-PCR) was used to determine the relative mRNA levels of *ABCB1*, *ABCC1* and *ABCG2* genes in all 70 samples. PCR reactions were performed with 100 ng of cDNA using TaqMan Gene Expression Assay methodology. Primers and probes specific for each ABC pump gene, and *HPRT* as a reference gene for the normalization of target mRNA expression, were obtained from Applied Biosystems as following Assay-on-Demand Gene Expression Products: *ABCB1* (Hs00324085_m1), *ABCC1* (Hs00219905_m1), *ABCG2* (Hs01053790_m1) and *HPRT1* (Hs01003267_m1).

PCR reactions were performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems, USA) according to the manufacturer's recommendations. Each sample was tested in triplicate in at least three independent experiments and mRNA expression levels in tumor samples were analyzed relative to corresponding non-tumor samples using 2-ΔΔCt method [16].

2.4 Immunohistochemistry

Tumor samples in paraffin blocks were cut in 2-3 µm sections for immunohistochemical analyses. Upon deparaffinization and antigen retrieval, the following antibodies were used for immunostaining according to the manufacturer's instructions: anti-P-gp ("ready to use", clone: JSB-1, Abcam, USA), anti-MRP1 (1 : 100, clone: MRPm5, Abcam, USA) and anti-BCRP (1 : 40, clone: BXP-21, Abcam, USA). The streptavidin-biotin immunostaining procedure was further performed (LSAB+ Kit, Peroxidase Labeling, K0690, DAKO Cytomation, Denmark). Antigen-antibody complexes were visualized with diaminobenzidinehydrochloride substrate solution (DAB, DAKO Cytomation, Denmark), while the cell nuclei were contra-stained with Mayer's hematoxylin. Positive immunoreactivity of P-gp in peripheral nerve, MRP1 in hepatocytes and BCRP in placental trophoblasts were used as the internal positive controls. Immunohistochemical (IHC) results were independently evaluated using a Leica DM2500 light microscope (Leica Microsystems, Germany) by two pathologists (JS and ZM) blinded to the clinicopathological data. Interpretation differences greater than 10% were discussed and solved by an agreement between examiners.

The expression of ABC transporter proteins was evaluated according to the scoring methodology previously described [17, 18, 19]. Pump protein expression in more than 10% of malignant cells, at each tissue section, was considered as positive, while ≤ 10% of the stained tumor cells were considered negative. The individual

Table 1. mRNA expression of ABC transporters in NSCLC samples and their association with neoadjuvant chemotherapy.

Expression status (NT vs. T)		non-NACT NP ^a (%)	NACT NP (%)	P value
<i>ABCB1</i>	Yes ^b	24 (70.6)	21 (60.0)	0.45
	No ^c	10 (29.4)	14 (40.0)	
<i>ABCC1</i>	Yes	15 (44.2)	14 (40.0)	0.81
	No	19 (55.8)	21 (60.0)	
<i>ABCG2</i>	Yes	29 (87.9)	25 (71.4)	0.13
	No	4 (12.1)	10 (28.6)	

^aNP, number of patients per group.

^b Downregulated expression, significantly decreased mRNA level by at least 50% in tumor (T) compared to matching non-tumor (NT) samples.

^c All other expression level relations between T and NT.

Table 2. Protein expression of ABC transporters in NSCLC samples and their association with neoadjuvant chemotherapy.

Positive expression ^a		non-NACT NP ^b (%)	NACT NP (%)	P value
P-gp	Yes	21 (60.0)	20 (57.2)	1
	No	14 (40.0)	15 (42.8)	
MRP1	Yes	32 (91.4)	24 (68.6)	0.03^c
	No	3 (8.6)	11 (31.4)	
BCRP	Yes	18 (51.4)	20 (57.2)	0.8
	No	17 (48.6)	15 (42.8)	

^aPositive expression, more than 10% of immunopositive tumor cells.

^bNP, number of patients per group.

^c Bold indicates statistically significant value, $P < 0.05$.

expression and co-expression of all proteins was examined.

2.5 Statistical analysis

Significant differences were determined by statistical package R (version 3.1.3 (2015-03-09) Copyright (C) 2015 The R Foundation for Statistical Computing). Paired t-test was used to evaluate statistical differences in mRNA expression between matched tumor and non-tumor samples of each patient. mRNA expression in tumor samples was considered to be downregulated if its level was significantly decreased by at least 50% compared to non-tumor samples. Conversely, differences in relative mRNA levels between total tumor and non-tumor samples in each group (NACT and non-NACT) were calculated using unpaired t-test. Fisher exact test was used to analyze the association between mRNA/protein expression and NACT status. Survival analyses were performed by the Kaplan & Meier product-limit method. The log rank test was used to evaluate the significant difference between pairs of survival probabilities. Overall survival rate was calculated from day one after surgery to the last follow-up examination or patient's death. Statistical differences were considered significant when $P < 0.05$.

3. Results

3.1 ABC transporter mRNA expression analysis

The expression levels of *ABCB1*, *ABCC1* and *ABCG2* mRNAs in all samples were determined by qRT-PCR. In the group without NACT, overall expression of *ABCB1* and *ABCG2* in tumor samples was significantly lower than in non-tumor samples (Fig. 1A, $P < 0.001$). Similarly, *ABCB1* and *ABCG2* mRNA expression was significantly downregulated in NSCLC samples compared to non-tumor samples in the NACT group (Fig. 1B, $P < 0.001$). *ABCB1* expression was downregulated in 70%, *ABCC1* in 44% and *ABCG2* in 88% of NSCLC samples compared to matched non-tumor samples in the non-NACT group (Table 1). However, the downregulated expression of ABC pumps in the NACT samples had lower frequency compared to non-NACT samples but did not reach statistical significance (Table 1). Specifically, decreased *ABCB1*, *ABCC1* and *ABCG2* mRNA levels were detected in 60%, 40% and 71% of NSCLC samples with NACT, respectively (Table 1).

3.2 Protein expression analysis of ABC transporters

Protein expression of ABC transporters was evaluated using immunohistochemical staining (Fig. 1C). Positive protein expression of the MDR efflux pumps was present in more than 50% of samples in both groups (Table 2). In the group without NACT, positive P-gp expression was observed in 60% of samples, showing a trend of decrease to 57% in the NACT group. Positive MRP1 expression was detected in 91% of non-NACT samples and was significantly decreased to 69% in the NACT group (Table 2, $P = 0.03$). The incidence of BCRP positive samples increased from 51% in non-NACT group to 57% in NACT group but did not reach statistical significance.

3.3 Co-expression of ABC transporters in NSCLC patients in relation to NACT status

The presence of co-expressed ABC transporters was further evaluated (Table 3). Almost all patients without NACT had positive expression of at least one ABC pump (97%) and this percentage decreased in the NACT group (86%). The frequency of samples with positive expression of only one MDR efflux transporter was identical in both groups (23%). However, a positive expression of only MRP1 in the group without NACT (75%) was considerably decreased in the NACT group (43%). Conversely, BCRP, whose sole expression was not detected in the group without NACT, appeared among NSCLC samples in the NACT group (29%). Unlike MRP1 and BCRP, the single occurrence of P-gp expression did not differ considerably between the groups (Table 3).

The incidence of positive co-expression of any two pumps was 44% in the group without NACT and slightly decreased in the NACT group (40%). Double positive expression of P-gp/MRP1 and MRP1/BCRP was the most prevalent in the group without NACT (53% and 47%, respectively). However, the incidence of these samples decreased in the NACT group (42% and 41%, respectively). Double positive P-gp/BCRP expression which was not present in non-NACT group, was detected in NACT samples (17%). Co-expression of all three transporters was more frequently present in the NACT group (37%) than in the non-NACT group (32%).

3.4 The impact of ABC transporter expression on the survival of patients who received neoadjuvant therapy

Survival rates indicated that the interaction of P-gp and NACT significantly influenced patient survival (Fig. 2A, $P = 0.034$).

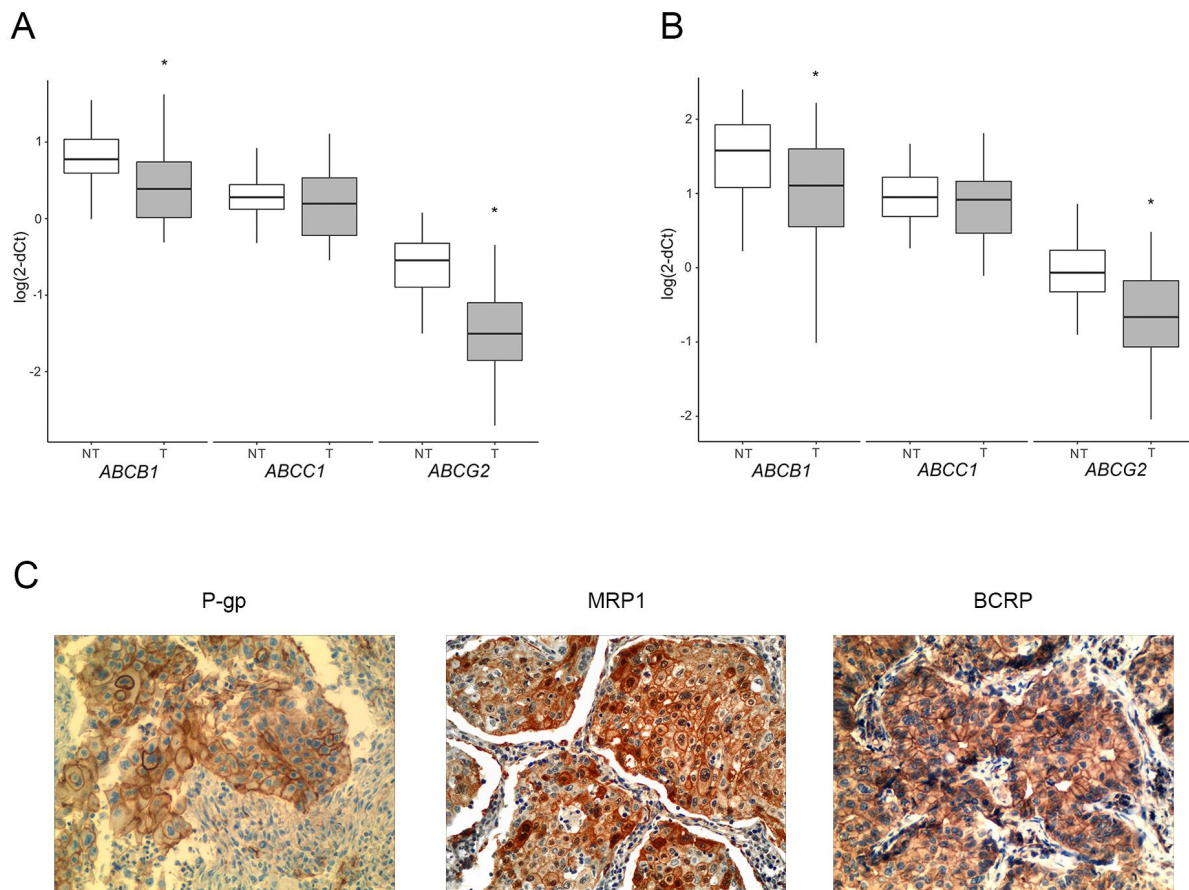


Figure 1. ABC transporters expression. Box plot analysis of *ABCB1*, *ABCC1* and *ABCG2* mRNA expression levels in primary tumor (T) and matched non-tumor tissue (NT) samples in non-NACT (A) and NACT (B) group of samples. Results are presented as log₁₀ of each mRNA expression relative to *HPRT* mRNA level. * indicates $p < 0.05$ as significantly decreased mRNA level in tumor samples compared to non-tumor control samples. Immunohistochemical analysis of P-gp, MRP1 and BCRP protein expression in NSCLC specimens (C). Representative examples of P-gp expression on the surface of the cells (20 × objective), heterogeneous MRP1 expression with linear expression on the cell membrane and diffuse expression in the cytoplasm of the malignant cells (20 × objective) and BCRP expression on the surface of the cells (20 × objective).

Specifically, the patients who did not express P-gp and did not receive NACT, had a significantly worse prognosis than patients without P-gp expression who received NACT ($P = 0.019$). MRP1 and BCRP expression did not significantly influence the survival of patients who received NACT (Fig. 2B and 2C, $P = 0.13$ and $P = 0.11$, respectively).

4. Discussion

Previous clinical trials have shown some benefits of NACT administered to NSCLC patients [20]. However, the impact of this approach was poorly evaluated in relation to molecular alterations linked to disease pathogenesis and response to therapy, including alterations in drug resistance-associated transporters. In the present study, we assessed the impact of NACT on mRNA and protein expression of P-gp, MRP1 and BCRP in NSCLC. Moreover, we then determined their potential role as molecular markers of chemosensitivity and prognosis of NSCLC patients. Our results suggest that determination of the ABC transporter expression status can help in predicting the sensitivity to planned adjuvant therapy and selecting patients who will benefit from NACT treatment.

Analyses of mRNA expression in the groups with and without administered NACT, revealed that mRNA levels of *ABCB1* and *ABCG2* were significantly lower in tumors compared to cognate non-tumor tissues, whereas *ABCC1* mRNA expression did not differ significantly between cancer and surrounding non-tumor lung tissue, in both groups. Similar findings were previously reported for *ABCB1* and *ABCG2* expression in NSCLC samples without NACT treatment [21, 22, 23, 24]. On the other hand, Li and colleagues observed increased *ABCC1* expression in tumor tissues of non-NACT samples [22]. However, no published data were available for tumor specimens from patients who received NACT. The current study shows, for the first time, the same pattern of ABC transporters' gene expression in non-tumor and tumor tissues of both investigated groups (i.e. with and without NACT).

It is not surprising that fast growing NSCLC cells have down-regulated *ABCB1* and *ABCG2* expression in comparison with surrounding non-tumor tissue. Namely, *ABCB1* and *ABCG2* are considered as stemness markers, usually present in quiescent cells [25] Therefore, their expression is decreased in actively dividing NSCLC cells, while in non-tumor cells these genes sustain the ex-

Table 3. Incidence of co-expression of ABC transporters in NSCLC patient specimens with or without NACT treatment.

	non-NACT NP ^a	NACT NP
Total NP	35	35
Protein expression		
Negative	1 (3%) ^b	5 (14%) ^b
Positive	34 (97%) ^b	30 (86%) ^b
Positive expression of only 1 protein	8 (23%) ^c	7 (23%) ^c
P-gp	2 (25%) ^d	2 (28%) ^d
MRP1	6 (75%) ^d	3 (43%) ^d
BCRP	0 (0%) ^d	2 (29%) ^d
Positive expression of any 2 proteins	15 (44%) ^c	12 (40%) ^c
P-gp /MRP1	8 (53%) ^e	5 (42%) ^e
P-gp/BCRP	0 (0%) ^e	2 (17%) ^e
MRP1/BCRP	7 (47%) ^e	5 (41%) ^e
Positive expression of all 3 proteins		
P-gp/MRP1/BCRP	11 (32%) ^c	11 (37%) ^c

^aNP, number of patients per group.

^b% of total number of patients.

^c% of total number of patients with positive protein expression.

^d% of total number of patients with positive expression of only 1 protein.

^e% of total number of patients with positive expression of any 2 proteins.

pression of transporters due to the necessity of their physiological role in the elimination of xenobiotics harmful to lungs [6]. The status of *ABCC1* expression differs due to the fact that its product MRP1 is present at the invasive front of the lung tumors, particularly in areas with lymphatic or blood vessels [6].

Another novelty of our study is the observed trend of decrease in the proportion of NSCLC to non-tumor tissue with downregulated gene expression of each drug efflux pump in the NACT group compared to non-NACT group. This result points at the impact of NACT on the overall increase in *ABCB1*, *ABCC1* and *ABCG2* expression in tumor tissue.

A particular focus of the current study was on the ABC transporter protein expression in the two groups of interest. Positive protein expression of all three MDR pumps was present in both non-NACT and NACT group with a substantial frequency. MRP1 was the predominant efflux pump expressed in NSCLC samples, both with and without NACT, which is in agreement with earlier publications on samples without preoperative treatment [14, 26, 27]. However, in our set of NSCLC samples, the incidence of MRP1 positive expression was significantly decreased in the NACT group regardless of the dominant use of carboplatin, while BCRP positive samples showed a trend of increase when compared to the non-NACT group. In contrast to our findings, Berger and colleagues reported P-gp, but not MRP1, overexpression in NSCLC samples with NACT, probably due to the therapy regimen which included P-gp inducers such as vinorelbine, vincristine and taxol [27]. Although the NACT regimen included cisplatin known as an MRP1 inducer, Xiang and colleagues did not observe statistical difference in MRP1 expression between matched NSCLC samples before and after NACT [28].

The use of platinum-based agents in chemotherapeutic treatment is generally known to induce MRP1 expression and its ad-

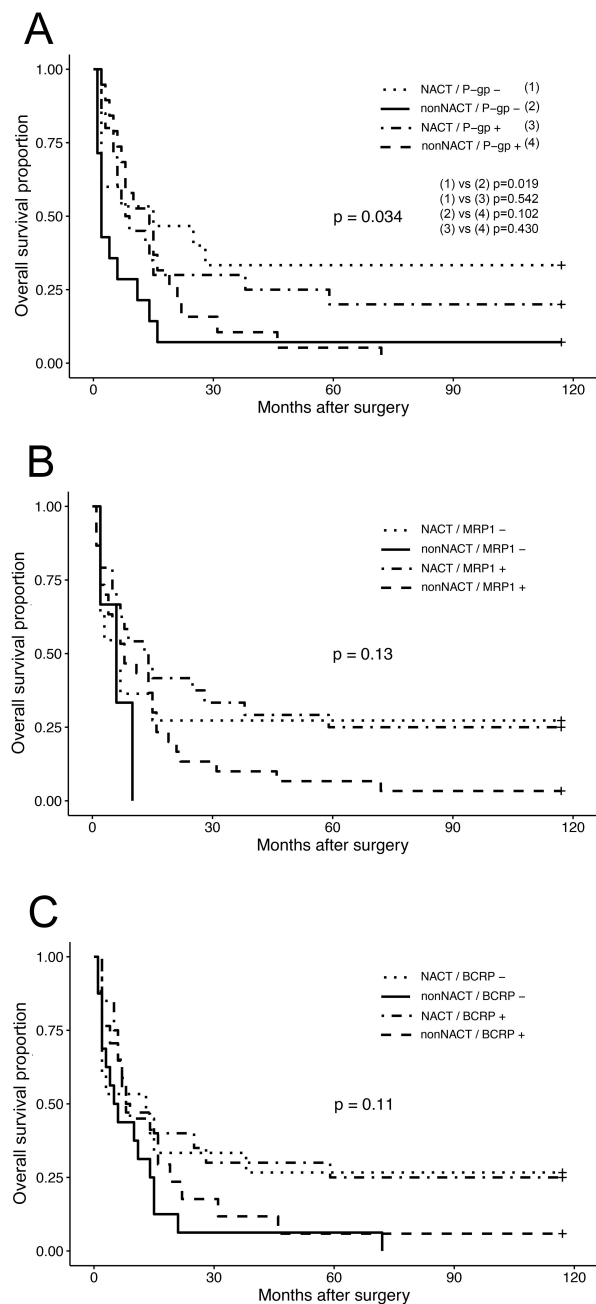


Figure 2. **Kaplan-Meier overall survival curves.** In general, P-gp expression and NACT status had a significant effect on patients' survival ($p = 0.034$) (A). Specifically, patients that did not express P-gp and received NACT had significantly better prognosis than patients devoid of P-gp expression who did not receive NACT treatment ($p = 0.019$), while other combinations of P-gp and NACT status did not show significant associations with patient prognosis. Moreover, MRP1 expression and NACT status (B), as well as BCRP expression and NACT treatment (C), did not significantly influence patients' survival.

ministration in NACT protocol would be expected to increase the incidence of MRP1 positive samples and lead to the development of drug resistance [6]. Conversely, we obtained opposite results

as MRP1 positive cells were eliminated and BCRP positive cells appeared to be selected upon platinum-based NACT approach. Although mechanistically these NACT effects on ABC pump expression are not clear and should be further investigated, the results obtained could have important clinical implications. They justify further use of platinum-based agents in adjuvant chemotherapy treatment in NSCLC patients that already received platinum-based NACT and suggest avoiding administration of BCRP drug substrates, such as methotrexate and tyrosine kinase inhibitors (for example gefitinib and erlotinib,) in the following adjuvant chemotherapy courses [29].

Importantly, we observed redistribution in the positive expression of each efflux pump and their combinations in NACT compared to non-NACT group. The frequency of positive MRP1 specimens and the co-presence with P-gp and BCRP was decreased in the NACT group, whereas the incidence of BCRP, P-gp/BCRP and triple pump positive samples was increased on the expense of the overall MRP1 decrease. To the best of our knowledge, this is the first study that comparatively evaluated individual expression and co-expression of all three MDR pumps with special emphasis on the effects of NACT in NSCLC. So far, only co-expression of P-gp and MRP1 was studied but without considering the effects on preoperative chemotherapy and without significant correlation between these pumps [26, 27, 30].

It is interesting to note that NACT had mainly opposite effects on gene expression and protein levels of the MDR transporters in our set of samples. NACT decreased the number of samples with positive protein expression, while increasing the frequency of samples with higher mRNA levels. Except for different methodologies used for protein and mRNA levels determination, we speculate that this effect of NACT could be a result of negative feedback loop between protein and gene expression. From a clinical point of view, this could have implications on the following courses of adjuvant therapy when we could expect increased protein expression and subsequent acquisition of resistance to chemotherapy, due to the presence of mRNA prepared to produce ABC transporters. Therefore, we believe that evaluating gene expression after NACT treatment could be important and useful for optimal scheduling of adjuvant chemotherapy in order to increase its efficacy and prevent the development of drug resistance.

Studies that evaluated the prognostic relevance of ABC transporter expression in NSCLC patients with NACT did not observe a significant association between P-gp, MRP1 and BCRP expression and patients' outcome [27, 31]. This is in concordance with our current findings on the effects of MRP1 and BCRP on survival of NSCLC in NACT and non-NACT groups. However, P-gp expression showed prognostic significance in these patients. Namely, patients without P-gp expression who received NACT had the most favorable prognosis compared to all other patients, particularly those without P-gp expression and without NACT treatment. P-gp expression was studied as a prognostic factor in chemotherapy naïve NSCLC patients in several studies [32, 33, 34], however our present study is the first to suggest its prognostic significance in relation to NACT treatment.

In conclusion, the present study showed that MRP1 and BCRP could be considered as NSCLC chemosensitivity biomarkers and could potentially be included in personalized therapy design in pa-

tients receiving NACT. We speculate that platinum-based NACT treatment eliminates MRP1 positive cells and has tendency to select BCRP positive cells, probably due to the individual differences among investigated patients. Moreover, the study revealed that P-gp negative expression had prognostic relevance and contributed to favorable prognosis in patients who received NACT. Although the results of the present study are novel and significant for potential implementation in clinical practice, the definite applicability of ABC transporters expression as potential molecular markers related to NACT treatment, should be further tested on larger cohorts of patients. Considering that the current clinical practice introduces NACT as a treatment strategy aimed to improve therapy response and survival of NSCLC patients, studying the expression status of MDR efflux transporters after NACT seems to be significant for making proper decisions about following adjuvant treatment of NSCLC patients.

Authors' contributions

JB and NT: study design; TS, JS, ZM, ZM, VM, SSB and JB: performing the experiments; all authors: data elaboration and interpretation; TS and MD: statistical analysis; TS, MP and JB: paper writing; JS and MD: preparing the figures; all authors: final editing and approval of the manuscript in its present form.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under Grant number III41031.

Conflicts of interest

The authors declare no competing interests.

Submitted: September 25, 2019

Accepted: November 28, 2019

Published: December 20, 2019

References

- [1] Gridelli C, Rossi A, Carbone D, Guarize J, Karachaliou N, Mok T, *et al.* Non-small-cell lung cancer. *Nat Rev Dis Prim* 2015; 1: 15009.
- [2] Zarogoulidis K, Zarogoulidis P, Darwiche K, Boutsikou E, Machairiotis N, Tsakiridis K, *et al.* Treatment of non-small cell lung cancer (NSCLC). *J Thorac Dis* 2013; 5(S4): S389–S396.
- [3] McElnay P, Lim E. Adjuvant or neoadjuvant chemotherapy for NSCLC. *J Thorac Dis* 2014; 6(S2): S224–S227.
- [4] Xia W, Yu X, Mao Q, Xia W, Wang A, Dong G, *et al.* Improvement of survival for non-small cell lung cancer over time. *Oncol Targets Ther* 2017; 10: 4295–4303.
- [5] Sun YL, Patel A, Kumar P CZ. Role of ABC transporters in cancer chemotherapy. *Chin J Cancer* 2012; 31: 51–57.
- [6] van der Deen M, de Vries EGE, Timens W, Scheper RJ, Timmer-Bosscha H, Postma DS. ATP-binding cassette (ABC) transporters in normal and pathological lung. *Respir Res* 2005; 6: 59.
- [7] Pesic M, Markovic JZ, Jankovic D, Kanazir S, Markovic ID, Rakic L, *et al.* Induced resistance in the human non small cell lung carcinoma (NCI-H460) cell line in vitro by anticancer drugs. *J Chemother* 2006; 18: 66–73.
- [8] Young LC, Campling BG, Cole SPC, Deeley RG, Gerlach JH. Multidrug Resistance Proteins MRP3, MRP1, and MRP2 in Lung Cancer. *Clin Cancer Res* 2001; 7: 1798–1804.
- [9] Tang Y, Hou J, Li G, Song Z, Li X, Yang C, *et al.* ABCG2 regulates the pattern of self-renewing divisions in cisplatin-resistant non-small cell lung cancer cell lines. *Oncol Rep* 2014; 32: 2168–2174.

- [10] Abe Y, Ohnishi Y, Yoshimura M, Ota E, Ozeki Y, Oshika Y, *et al.* P-glycoprotein-mediated acquired multidrug resistance of human lung cancer cells in vivo. *Br J Cancer* 1996; 74: 1929–1934.
- [11] Merk J, Rolff J, Dorn C, Leschber G, Fichtner I. Chemoresistance in non-small-cell lung cancer: can multidrug resistance markers predict the response of xenograft lung cancer models to chemotherapy? *Eur J Cardio-Thoracic Surg* 2011; 40: e29–e33.
- [12] Li J, Li ZN, Du YJ, Li XQ, Bao QL, Chen P. Expression of MRP1, BCRP, LRP, and ERCC1 in advanced non-small-cell lung cancer: Correlation with response to chemotherapy and survival. *Clin Lung Cancer* 2009; 10: 414–421.
- [13] Filipits M, Haddad V, Schmid K, Huynh A, Dunant A, André F, *et al.* Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program. *Clin Cancer Res* 2007; 13: 3892–3898.
- [14] Yoh K, Ishii G, Yokose T, Minegishi Y, Tsuta K, Goto K, *et al.* Breast Cancer Resistance Protein Impacts Clinical Outcome in Platinum-Based Chemotherapy for Advanced Non-Small Cell Lung Cancer. *Clin Cancer Res* 2004; 10: 1691–1697.
- [15] Stojic J, Stankovic T, Stojkovic S, Milinkovic V, Dinic J, Milosevic Z, *et al.* Prolonged survival after neoadjuvant chemotherapy related with specific molecular alterations in the patients with non-small cell lung carcinoma. *Exp Mol Pathol* 2015; 98: 27–32.
- [16] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Med Oncol* 2001; 25: 402–408.
- [17] Ota S, Ishii G, Goto K, Kubota K, Kim YH, Kojika M, *et al.* Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy. *Lung Cancer* 2009; 64: 98–104.
- [18] Wei H, Lu W, Li M, Zhang Q, Lu S. Concomitance of P-gp/LRP expression with EGFR mutations in exons 19 and 21 in non-small cell lung cancers. *Yonsei Med J* 2016; 57: 50–57.
- [19] Rybárová S, Hodorová I, Mihalik J, Mirossay L. MRP1 and GSTp1 expression in non-small cell lung cancer does not correlate with clinicopathological parameters: A Slovakian population study. *Acta Histochem* 2014; 116: 1390–1398.
- [20] Salvà F, Felip E. Neoadjuvant chemotherapy in early-stage non-small cell lung cancer. *Transl Lung Cancer Res* 2013; 2: 398–402.
- [21] Abe Y, Nakamura M, Ota E, Ozeki Y, Tamai S, Inoue H, *et al.* Expression of the Multidrug Resistance Gene (MDR1) in Non-small Cell Lung Cancer. *Japanese J Cancer Res* 1994; 85: 536–541.
- [22] Li X, Li J, Shi S, Chen P, Yu L, Bao Q. Expression of MRP1, BCRP, LRP and ERCC1 as prognostic factors in non-small cell lung cancer patients receiving postoperative cisplatin-based chemotherapy. *Int J Biol Markers* 2009; 24: 230–237.
- [23] Mao Y, Doyle A, Yang W, Wei Y, Krasna MJ, Ross DD. Expression of BCRP gene in the normal lung tissue and lung cancer. *Chinese J Cancer Res* 2001; 13: 22–26.
- [24] Vesel M, Rapp J, Feller D, Kiss E, Jaromi L, Meggyes M, *et al.* ABCB1 and ABCG2 drug transporters are differentially expressed in non-small cell lung cancers (NSCLC) and expression is modified by cisplatin treatment via altered Wnt signaling. *Respir Res* 2017; 18: 52.
- [25] Lathia JD, Liu H. Overview of Cancer Stem Cells and Stemness for Community Oncologists. *Target Oncol* 2017; 12: 387–399.
- [26] Roy S, Kenny E, Kennedy S, Larkin A, Ballot J, Perez De Villarreal M, *et al.* MDR1/P-glycoprotein and MRP-1 mRNA and protein expression in non-small cell lung cancer. *Anticancer Res* 2007; 27: 1325–1330.
- [27] Berger W, Setinek U, Hollaus P, Zidek T, Steiner E, Elbling L, *et al.* Multidrug resistance markers P-glycoprotein, multidrug resistance protein 1, and lung resistance protein in non-small cell lung cancer: Prognostic implications. *J Cancer Res Clin Oncol* 2005; 131: 355–363.
- [28] Xiang F, Yu W, Shen Y, Wu C, Wang Y. Effects of neoadjuvant chemotherapy on the quantitative expression of P-gp, LRP, MRP, GST- π in NSCLC and its clinical significance. *Zhongguo Fei Ai Za Zhi* 2007; 10: 398–405.
- [29] Noguchi K, Katayama K, Sugimoto Y. Human ABC transporter ABCG2/BCRP expression in chemoresistance: Basic and clinical perspectives for molecular cancer therapeutics. *Pharmacogenomics and Personalized Medicine* 2014; 7: 53–64.
- [30] Paredes Lario A, Blanco García C, Echenique Elizondo M, Lobo C. Expresión de proteínas relacionadas con resistencia a múltiples fármacos y resistencia a la quimioterapia en el cáncer de pulmón. *Arch Bronconeumol* 2007; 43: 479–484.
- [31] Shien K, Toyooka S, Ichimura K, Soh J, Furukawa M, Maki Y, *et al.* Prognostic impact of cancer stem cell-related markers in non-small cell lung cancer patients treated with induction chemoradiotherapy. *Lung Cancer* 2012; 77: 162–167.
- [32] Liu Z, Sun R, Zhang J, Ding C, Ma G, Zhao X, *et al.* Prognostic value of MRP, LRP and P-gp in patients with non-small cell lung cancer. *Int J Clin Exp Pathol* 2017; 10: 6799–6808.
- [33] Maraz A, Furak J, Palfoldi R, Eller J, Szanto E, Kahan Z, *et al.* Roles of BCL-2 and MDR1 expression in the efficacy of paclitaxel-based lung cancer chemoradiation. *Anticancer Res* 2011; 31: 1431–1436.
- [34] Zou F, Seike M, Noro R, Kunugi S, Kubota K, Gemma A. Prognostic significance of ABCB1 in stage I lung adenocarcinoma. *Oncol Lett* 2017; 14: 313–321.