

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The pharmacogenomics of vincristine-induced peripheral neuropathy in pediatric acute lymphoblastic leukemia patients in Serbia – a single center experience

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SUMMARY

Introduction/Objective Vincristine (VCR) is one of the key drugs in current treatment protocols for pediatric acute lymphoblastic leukemia (ALL). By destabilizing microtubules, VCR arrests cells in metaphase, inducing apoptosis of malignant cells. VCR also causes axonal degradation and impairment of axonal transport, which leads to VCR-induced peripheral neuropathy (VIPN).

This study aimed to investigate the association of five variants in pharmacogenes involved in VCR metabolism with VIPN in Serbian ALL children. We also wanted to discover candidate pharmacogenomic markers of VIPN in Serbian population.

Methods PCR and sequencing-based methodology was used to detect variants in *CYP3A5*, *CEP72*, *ACTG1*, *MIR3117*, and *MIR4481* genes. Statistical analyses were performed for investigating their association with VIPN in 56 pediatric ALL patients. Population VCR pharmacogenomics analysis of 17 pharmacogenes from in-house next-generation sequencing data was also done. Data on allele frequency distribution for the European population were extracted from public databases.

Results During the treatment, 17.86% of patients developed VIPN. Association analyses have shown that none of the genetic variants contributed to the occurrence of VIPN in our study. Population pharmacogenomics study did not reveal valid candidate pharmacovariants for VIPN. Our results suggested that pre-emptive pharmacogenetic testing for VCR is not applicable presently.

Conclusion More comprehensive approaches are needed to identify the panel of genes that could explain the VIPN development after VCR administration in ALL patients. Utilizing better designed genome-wide association studies and more robust artificial intelligence-based tools would provide a panel of pharmacogenes for pre-emptive tests of VIPN to individualize therapy for ALL in children.

Keywords: acute lymphoblastic leukemia; pharmacogenomics; vincristine; vincristine-induced peripheral neuropathy (VIPN)

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising about one-fourth of all cancers in children. The cure rate for childhood ALL reached 85%, but about 75% of all patients experience treatment side effects, and 1–3% of all children with ALL die due to the treatment toxicity [1]. Application of the principles of pharmacogenomics could lower the number and intensity of drug-induced side effects.

One of the key drugs in treatment protocols for pediatric ALL is vincristine (VCR). VCR binds to tubulin, preventing the polymerization of microtubules and inducing apoptosis in cancer cells. However, the affinity of VCR for tubulin makes the microtubules in nerve fibers a likely target of VCR action, leading to axonal degradation, impairing axonal transport, and causing the development of VCR-induced peripheral neuropathy (VIPN). VIPN is a major

side effect of VCR administration, manifesting as muscle weakness, areflexia, neuropathic pain, sensory loss, or autonomic polyneuropathies [2]. VIPN often results in dose reduction, treatment delays, and further withdrawal. The occurrence of VIPN in children is determined by multiple factors [3, 4], with most of the recent studies focusing on genetic influences [5].

The most comprehensive candidate gene and genome-wide association studies (GWAS) pointed to the possible involvement of following pharmacogenes: *CYP3A5*, *CEP72*, *ACTG1* [6–10]. Also, several variants in genes encoding miRNAs were shown to be potential pharmacogenomic markers of VIPN [11]. *CYP3A5* is the most important metabolizer of VCR. The rs776746 variant in the third intron introduces a premature stop codon which leads to low or no expression of this enzyme [6]. *CEP72* gene encodes a centrosomal protein important for microtubule formation and stability of the centrosome. Variant rs924607 in the promoter region

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of this gene could be associated with a higher risk of development and severity of VIPN [11], by possibly introducing a binding site for transcriptional repressor NKX-6.3, lowering expression on *CEP72* mRNA [7]. Alterations in the interactions between the gamma isoform of actin (*ACTG1*) and microtubules are involved in signal transduction to the actin cytoskeleton. The variant *ACTG1* rs1135989 was associated with higher risk and more frequent episodes of high-grade neurotoxicity, as well as a lower tolerated VCR dose [10]. A recent study identified variants in genes encoding miRNAs related to VIPN [11]. The variant *MIR3117* rs12402181 reduces the risk of VIPN by decreasing the degradation and increasing the expression of *ABCC1* and *RALBP1* mRNAs, thus increasing the efflux of VCR from the axons. The variant *MIR4481* rs7896283 increases the stability of the premature miR-4481, lowering expression of proteins in the axon guidance pathway that may affect peripheral nerve regeneration [11]. Several studies have indicated additional pharmacogenes potentially involved in the development of VIPN [8, 9, 12–15].

The aim of this study was to determine if the selected genetic variants *CYP3A5* rs776746, *CEP72* rs924607, *ACTG1* rs1135989, *MIR3117* rs12402181, and *MIR4481* rs7896283 are associated with the development of VIPN in ALL children treated with VCR in Serbia. Additionally, we aimed to perform an analysis of clinical exome sequencing data for population pharmacogenomics study to discover candidate pharmacogenomic markers of VIPN in the Serbian population.

METHODS

Subjects

This study included 56 children diagnosed with ALL between 2010 and 2018 at the University Children's Hospital, Belgrade, Serbia. It was approved by the University Children's Hospital Ethics Committee and performed according to the Declaration of Helsinki. Informed consent was obtained from the parents or legal guardians of each patient.

All patients were treated according to the ALL Intercontinental Berlin-Frankfurt-Munster (IC-BFM) 2009 protocol, divided into the usual phases: remission induction and early intensification, consolidation, reinduction and maintenance. The patients were stratified into three risk groups: standard risk (SR), intermediate risk (IR), and high risk (HR). Patients in the IR and HR groups were randomized during the early intensification phase in arm 1 (IR-1 and HR-1) and arm 2 (IR-2 and HR-2). Stratification and randomization of the patients resulted in patients receiving different number of VCR doses (1.5 mg/m² per dose) during the treatment (Table 1) [16].

The patients were assessed for VIPN using the National Cancer Institute Common Toxicity Criteria [17]. A patient was diagnosed with VIPN if exhibiting one or more symptoms of VCR-related neurotoxicity at the end of the reinduction phase.

Data for the European population frequencies of the investigated variants were extracted from the Genome Aggregation Database, GnomAD.

Genetic variants detection

Detection of variants investigated in this study (*ACTG1* rs1135989, *CEP72* rs924607, *MIR3117* rs12402181, *MIR4481* rs7896283, and *CYP3A5* rs776746) was performed using PCR and sequencing-based methodology as described elsewhere [18]. Primer sequences and annealing conditions used for amplification of each variant are available upon request. Allele frequency for *CEP72* rs924607 in healthy controls of Serbian descent was determined using the same methodology.

Population pharmacogenomics study

We have searched the literature on the PubMed database before April 2021 using the terms “vincristine” AND “pharmacogenomics” OR “pharmacogenetics” AND “GWAS” OR “candidate gene” OR “vincristine-induced peripheral neuropathy.” Studies were identified from the titles and abstracts by the primary (BR) and the secondary reviewer (BZ).

In-house database of 154 TruSight One Illumina sequenced clinical exomes belonging to individuals of Serbian descent was used to search for variants in 17 genes found during literature search that could be related to VCR metabolism: *CYP3A4*, *CYP3A5*, *ABCB1*, *PON1*, *ABCA4*, *ABCG1*, *CY51A1*, *SLCO1C1*, *ABCC1*, *SLC5A7*, *TTPA*, *ABCC2*, *SYNE2*, *COCH*, *TUBB1*, *TUBB2B*, and *TUBB3*.

The criteria for pharmacogenomics relevance of a variant were allele frequency higher than 5% and assigned annotation in PharmGKB database [19].

Assignment of a Level of Evidence by the PharmGKB annotation scoring system for clinical and variant annotations enables easier identification of significant pharmacovariants. The clinical annotation score represents the sum of the scores of all attached variant, guideline, and drug label annotations. Variant annotations are scored depending on the following: phenotype category, p-value, cohort size, effect size, and weighting by study type or by association and significance [19].

Statistical analysis

All variants were tested for the Hardy–Weinberg equilibrium (HWE) using an exact test.

The association of age of the patients and occurrence of VIPN was tested using logistic regression. The associations of immunophenotype, sex, and the number of doses administered were tested for association with VIPN using Fisher's exact test.

The association between the genotyped variants and VIPN was tested using the multiplicative genetic model. Multivariate analysis was adjusted for the number of VCR doses or sex.

Table 1. Characteristics of pediatric acute lymphoblastic leukemia patients

Characteristics	Patients without VIPN	Patients with VIPN	Total	p ¹
Age (years)				0.913 ²
Average	7	6.8	7	
Median	5.5	3.7	5.3	
Range	0.7–17.9	1.0–17.1	0.7–17.9	
Sex (n/%)				0.171 ³
Male	26 (56.5%)	3 (30%)	29 (51,8%)	
Female	20 (43.5%)	7 (70%)	27 (49,2%)	
Immunophenotype [n (%)]				1 ³
B-lineage	42 (91.3%)	10 (100%)	52 (49,2%)	
T-lineage	4 (8.7%)	0 (0%)	4 (7,1%)	
Risk group [n (%)]				0.346 ³
SR; 8 VCR doses	10 (21.7%)	1 (10%)	12 (21.4%)	
IR-1; 8 VCR doses	18 (39.1%)	2 (20%)	19 (33.9%)	
IR-2; 12 VCR doses	5 (10.9%)	3 (30%)	8 (14.3%)	
HR-1; 12 VCR doses	10 (21.7%)	3 (30%)	13 (23.2%)	
HR-2; 16 VCR doses	3 (6.5%)	1 (10%)	4 (7.2%)	

VIPN – vincristine-induced peripheral neuropathy; VCR – vincristine; SR – standard risk; IR – intermediate risk; HR – high risk;
¹p-value refers to statistical testing the difference between groups of patients with and without VIPN;
²Logistic regression;
³Fisher's exact test

Association analyses were performed using SPSS (version 21, IBM, Armonk, NY, USA).

Probabilities lower than 5% were considered statistically significant.

RESULTS

Demographic and clinical characteristics of the subjects

This study encompassed 56 children with ALL, with slight predominance of male sex (n = 29; 51.8%). The median age at diagnosis was 5.3 years, ranging 0.7–17.9 years. Risk stratification revealed the following distribution: SR – 11 (19.6%), IR – 28 (50%), and HR – 17 (30.4%).

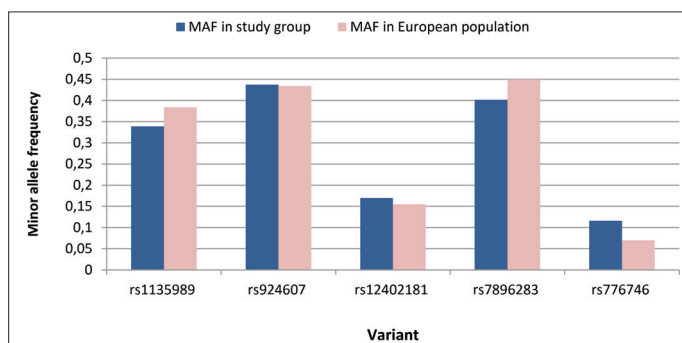


Figure 1. Minor allele frequencies (MAFs) of analyzed genetic variants in the study group and European population; all investigated variants were in Hardy-Weinberg equilibrium (HWE) (for rs1135989 HWE was 0.768, for rs924607 it was 0.790, and for the other variants 1); the data for MAF in the European population was extracted from the GnomAD database

Ten patients (17.86%) developed VIPN during the therapy, with high predominance of girls (70%). The median age at diagnosis of the patients that developed VIPN was 3.7 years, ranging 1–17.1 years. Nine out of ten patients were stratified into the IR and HR groups (IR – 5, HR – 4).

Occurrence of VIPN was higher in girls, though statistical significance has not been demonstrated (0.171, Fisher's exact test, OR = 3.03). A trend of higher occurrence of VIPN in patients who received more VCR doses was observed, but without statistical significance. However, patients who received more than 10 doses of VCR were 3.6 times more likely to develop VIPN than patients who received less than 10 doses (OR = 0.363; CI = 0.83–15.89; p = 0.092; Fisher's exact test) (Table 1).

Association of pharmacogenetic markers with VIPN

The HWE testing showed that genotype frequencies corresponding to all variants investigated in this study were in equilibrium. Frequencies of investigated variants in the Serbian pediatric ALL patients and the control group of European origin and results of HWE testing are presented in Figure 1.

We analyzed the association between the investigated variants and VIPN. For each variant, we evaluated the contribution associated with each additional minor allele to the probability of developing the neuropathy using a multiplicative genetic model [20]. In univariate analysis, no variant showed a statistically significant association with the VIPN. Applying logistic regression, the following p-values associated with variants in *ACTG1*, *CEP72*, *MIR3117*, *MIR4481*, and *CYP3A5* genes were obtained: 0.917, 0.898, 0.788, 0.310, and 0.577, respectively. In univariate or multivariate analysis (adjusted for the number of VCR doses or sex), no variant showed a statistically significant association with VIPN (Table 2).

Population pharmacogenomics study

In-house database of clinical exome sequences of 154 Serbian individuals was searched for variants in 17 genes with possible influence to VCR metabolism. Ten variants in six genes have been detected with allele frequency higher than 5%. Allele frequencies for most of them were similar to the ones detected in the European population (Table 3).

Only two variants were worthy to be further analyzed as potential pharmacogenetic markers of VIPN in the Serbian population. Although a variant *CYP3A4* rs4986910 is assigned to have a 2A level of evidence, its allele frequency in the Serbian population is very low (0.97%), similarly to European populations (0.73%). Therefore, it is not considered to be an appropriate candidate for pre-emptive pharmacogenomic testing.

Variant *CEP72* rs924607, considered to be the best candidate pharmacogenomic marker for VCR, is present in the Serbian population with 60% compared to 43.4% in European populations.

Table 2. Association of analyzed variants and vincristine-induced peripheral neuropathy (VIPN)

Gene	dbSNP	Genotype	n (%)	Patients without VIPN	Patients with VIPN	p	p ¹	p ²
<i>ACTG1</i>	rs1135989 C>T	CC	25 (44.6%)	21 (45.7%)	4 (40%)	0.917	0.837	0.886
		CT	24 (42.9%)	19 (41.3%)	5 (50%)			
		TT	7 (12.5%)	6 (13%)	1 (10%)			
<i>CEP72</i>	rs924607 C>T	CC	17 (30.4%)	14 (30.4%)	3 (30%)	0.898	0.909	0.791
		CT	29 (51.8%)	24 (52.2%)	5 (50%)			
		TT	10 (17.9%)	8 (17.4%)	2 (20%)			
<i>MIR3117</i>	rs12402181 G>A	GG	38 (67.9%)	31 (67.4%)	7 (70%)	0.788	0.963	0.605
		GA	17 (30.4%)	14 (30.4%)	3 (30%)			
		AA	1 (1.8%)	1 (2.2%)	0 (0%)			
<i>MIR4481</i>	rs7896283 T>C	TT	20 (35.7%)	15 (32.6%)	5 (50%)	0.310	0.188	0.500
		TC	27 (48.2%)	23 (50%)	4 (40%)			
		CC	9 (16.1%)	8 (17.4%)	1 (10%)			
<i>CYP3A5</i>	rs776746 A>G	AA	0 (0%)	0 (0%)	0 (0%)	0.577	0.702	0.360
		AG	13 (23.2%)	10 (21.8%)	3 (30%)			
		GG	43 (76.8%)	36 (78.2%)	7 (70%)			

¹Adjusted for the number of vincristine doses;²adjusted for sex**Table 3.** Allele frequencies of pharmacogenes related to vincristine-induced peripheral neuropathy in Serbian population (MAF > 5%)

Gene	dbSNP ¹	PharmGKB LoE ²	MAF in Serbian population (%)	MAF in European population (GnomAD) (%)
<i>CEP72</i>	rs924607	3	60	43.3
<i>ABCC2</i>	rs3740066	3	27.27	37.02
<i>ABCC2</i>	rs2273697	3	17.85	19.75
<i>ABCC2</i>	rs17222723	3	5.19	5.61
<i>SLC5A7</i>	rs1013940	VA	8.44	8.01
<i>PON1</i>	rs854560	4	37.01	36.7
<i>PON1</i>	rs662	3	22.4	28.06
<i>COCH</i>	rs1045644	VA	54.87	63.49
<i>TUBB1</i>	rs6070697	VA	15.26	17.94
<i>TUBB1</i>	rs463312	VA	6.17	5.26
<i>ABCC1</i>	rs246221	VA	31.82	30.5

MAF – minor allele frequencies; VA – variant annotation; LoE – level of evidence;

¹reference single nucleotide polymorphism (SNP) ID number (rs number) of SNPs that map an identical location assigned by the National Center for Biotechnology Information;²PharmGKB level of evidence, score of pharmacogenomics clinical (level 1: the highest, level 4: the lowest evidence association) and variant relevance

Despite high allele frequency in the Serbian population, this marker is not an applicable pharmacogenetic marker in pediatric ALL since our study demonstrated that it has the same distribution in pediatric ALL patients with and without VIPN.

DISCUSSION

Efforts towards treatment individualization of patients experiencing side effects of drugs are made constantly. Previous studies analyzed pharmacogenomics of some essential drugs used for treatment of pediatric ALL patients in Serbia [18, 21, 22].

We analyzed the correlation of five variants in pharmacogenes involved in VCR metabolism in pediatric ALL

patients with VIPN. A total of 56 patients treated according to the BFM protocol were included in the study. Ten patients (17.86%) developed VIPN during the treatment. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

Our results have shown a trend of higher occurrence of VIPN in girls as in several studies [7], while others reported no influence of patients' sex on VIPN development [12, 23]. We have also observed a trend of higher occurrence of VIPN in patients who received more than 10 doses of VCR during therapy, as they were 3.6 times more likely to develop VIPN. Several studies reported significant association between VCR dose and VIPN [7, 14], which is in contrast to some other studies [12, 24]. Assessment of cumulative dose effect of VCR to VIPN development has also shown conflicting results. There are reports of the absence of association between VIPN and cumulative VCR dose [24], as well as reports showing that cumulative dose of VCR is associated with VIPN [25].

Variant *CEP72* rs924607 was identified in GWAS study as key pharmacogene relevant for VIPN in ALL children [7], and those findings were confirmed in meta-analysis of pharmacogenomic data from over 500 patients [8]. However, several studies did not confirm this association [9, 23, 26]. Our results do not support the association of *CEP72* rs924607 with VIPN in pediatric ALL patients. The frequency of rs924607 T allele in our group of ALL patients with VIPN was almost the same as in ALL patients without VIPN (45% and 43.5%, respectively). The frequency of the same allele in our healthy population was rather high (60%), similar to frequency of this variant in the European population (43.4%). Therefore, we conclude that *CEP72* rs924607 pharmacomarker was not shown to be of pharmacogenomic relevance for the Serbian population.

Variants *ACTG1* rs1135989 and *CYP3A5* rs776746 have also been reported to contribute to VIPN susceptibility [6, 10]. Furthermore, analysis of the SNPs in miRNAs which could regulate VCR-related genes in a large cohort of pediatric ALL patients identified the *MIR3117* rs12402181 and *MIR4481* rs7896283 as variants significantly associated with VIPN [11]. In our study, none of the aforementioned

variants have shown statistically significant association with VIPN in pediatric ALL patients.

As additional variants have been indicated to contribute to the development of VIPN, we have performed the pilot population pharmacogenomics study in order to assess if the molecular genetics study of additional potential pharmacogenes would be beneficial and informative. The population pharmacogenomics study encompassed 17 relevant, literature-reviewed pharmacogenes present in in-house NGS database sequences of Serbian individuals: *CYP3A4*, *CYP3A5*, *ABCB1*, *PON1*, *ABCA4*, *ABCG1*, *CY51A1*, *SLCO1C1*, *ABCC1*, *SLC5A7*, *TTPA*, *ABCC2*, *SYNE2*, *COCH*, *TUBB1*, *TUBB2B*, and *TUBB3* [8, 9, 12–15]. Also, for these pharmacogenes our population pharmacogenomics study did not reveal valid candidate pharmacovariant for VIPN. A GWAS study identified *PNPLA3* rs735409 as potential pharmacovariant relevant for VCR use [27]. Further study confirmed that the *PNPLA3* rs735409 variant was associated with hepatotoxicity induced by asparagine administration [28], and we decided not to include it in our study. Our results have shown that pre-emptive pharmacogenetic testing for VCR is not presently applicable either in pediatric ALL patients or in patients of Serbian descent to whom VCR needs to be administered.

VCR metabolic pathway is complex. Many transporters and enzymes have an important role in the VCR pharmacokinetics and pharmacodynamics and so far, data have not shown a single universal VCR pharmacogenomic marker [29]. This is another example of the failure of predictions that have resulted from GWAS studies. A lack of consistency in neuropathy assessment, grading systems, and the choice of end points make it difficult to interpret results between studies [4, 29]. Treatment regimens, including number of VCR doses administered and lengths of VCR treatment, differ between treatment protocols used in different studies [30]. Furthermore, population-specific

genetic variants could have relevance or other expression quantitative trait loci of the genes in question for VIPN assessment.

The main limitation of our study is a small number of patients included. However, we have tried to overcome the limitations of GWAS studies by analyzing the patients treated with the same protocol and by establishing the comparative groups with similar VCR administration.

CONCLUSION

Our results have shown that pre-emptive pharmacogenetic testing for VCR is not presently applicable to neither pediatric ALL patients nor to patients of Serbian descent to whom VCR needs to be administered. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

More comprehensive approaches are needed to identify a panel of genes that could explain the VIPN development in ALL patients. Extending genome-wide research to larger, well-characterized and more diverse patient cohorts and development of more robust artificial intelligence bioinformatics tools, including machine learning, statistical learning, and soft-computing approaches, should be done. This would provide a panel of pharmacogenes that could be used for pre-emptive tests of VCR side effects leading to therapy individualization in pediatric ALL patients.

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REFERENCES

- Pavlovic S, Kotur N, Stankovic B, Zukic B, Gasic V, Dokmanovic L. Pharmacogenomic and Pharmacotranscriptomic Profiling of Childhood Acute Lymphoblastic Leukemia: Paving the Way to Personalized Treatment. *Genes* (Basel). 2019;10(3):191.
- van de Velde ME, Kaspers GL, Abbink FCH, Wilhelm AJ, Ket JCF, van den Berg MH. Vincristine-induced peripheral neuropathy in children with cancer: A systematic review. *Crit Rev Oncol Hematol*. 2017;114:114–30.
- Triarico S, Romano A, Attinà G, Capozza MA, Maurizi P, Mastrangelo S, et al. Vincristine-Induced Peripheral Neuropathy (VIPN) in Pediatric Tumors: Mechanisms, Risk Factors, Strategies of Prevention and Treatment. *Int J Mol Sci*. 2021;22(8):4112.
- Madsen ML, Due H, Ejkskjær N, Jensen P, Madsen J, Dybkær K. Aspects of vincristine-induced neuropathy in hematologic malignancies: a systematic review. *Cancer Chemother Pharmacol*. 2019;84(3):471–85.
- Pozzi E, Fumagalli G, Chiorazzi A, Canta A, Cavaletti G. Genetic factors influencing the development of vincristine-induced neurotoxicity. *Expert Opin Drug Metab Toxicol*. 2021;17(2):215–26.
- Egbelakin A, Ferguson MJ, MacGill EA, Lehmann AS, Topletz AR, Quinney SK, et al. Increased risk of vincristine neurotoxicity associated with low *CYP3A5* expression genotype in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2011;56(3):361–7.
- Diouf B, Crews KR, Lew G, Pei D, Cheng C, Bao J, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. *JAMA*. 2015;313(8):815–23.
- Wright GEB, Amstutz U, Drögemöller BI, Shih J, Rassekh SR, Hayden MR, et al. Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy Implicates Pharmacokinetic and Inherited Neuropathy Genes. *Clin Pharmacol Ther*. 2019;105(2):402–10.
- Li L, Sajdyk T, Smith EMLL, Chang CW, Li C, Ho RH, et al. Genetic Variants Associated With Vincristine-Induced Peripheral Neuropathy in Two Populations of Children With Acute Lymphoblastic Leukemia. *Clin Pharmacol Ther*. 2019;105(6):1421–8.
- Ceppi F, Langlois-Pelletier C, Gagné V, Rousseau J, Ciolino C, De Lorenzo S, et al. Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia. *Pharmacogenomics*. 2014;15(8):1105–16.
- Gutierrez-Camino Á, Umerez M, Martin-Guerrero I, García de Andoin N, Santos B, Sastre A, et al. Mir-pharmacogenetics of Vincristine and peripheral neurotoxicity in childhood B-cell acute lymphoblastic leukemia. *Pharmacogenomics J*. 2018;18(6):704–12.
- Lopez-Lopez E, Gutierrez-Camino A, Astigarraga I, Navajas A, Echebarria-Barona A, Garcia-Miguel P, et al. Vincristine pharmacokinetics pathway and neurotoxicity during early

- phases of treatment in pediatric acute lymphoblastic leukemia. *Pharmacogenomics*. 2016;17(7):731–41.
13. Abaji R, Ceppi F, Patel S, Gagné V, Xu CJ, Spinella JF, et al. Genetic risk factors for VIPN in childhood acute lymphoblastic leukemia patients identified using whole-exome sequencing. *Pharmacogenomics*. 2018;19(15):1181–93.
 14. Guilhaumou R, Solas C, Bourgairel-Rey V, Quaranta S, Rome A, Simon N, et al. Impact of plasma and intracellular exposure and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms on vincristine-induced neurotoxicity. *Cancer Chemother Pharmacol*. 2011;68(6):1633–8.
 15. Martin-Guerrero I, Gutierrez-Camino A, Echebarria-Barona A, Astigarraga I, Garcia de Andoin N, Navajas A, et al. Variants in vincristine pharmacodynamic genes involved in neurotoxicity at induction phase in the therapy of pediatric acute lymphoblastic leukemia. *Pharmacogenomics J*. 2019;19(6):564–9.
 16. ALL IC-BFM 2009. A Randomized Trial of the I-BFM-SG for the Management of Childhood non-B Acute Lymphoblastic Leukemia Final Version of Therapy Protocol from August-14-2009. 2015.
 17. COMMON TOXICITY CRITERIA MANUAL Common Toxicity Criteria, Version 2.0. 1999.
 18. Kotur N, Lazic J, Ristivojevic B, Stankovic B, Gasic V, Dokmanovic L, et al. Pharmacogenomic markers of methotrexate response in the consolidation phase of pediatric acute lymphoblastic leukemia treatment. *Genes (Basel)*. 2020;11(4):1–17.
 19. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther*. 2012;92(4):414–7.
 20. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. *Nat Protoc*. 2011;6(2):121–33.
 21. Gasic V, Zukic B, Stankovic B, Janic D, Dokmanovic L, Lazic J, et al. Pharmacogenomic markers of glucocorticoid response in the initial phase of remission induction therapy in childhood acute lymphoblastic leukemia. *Radiol Oncol*. 2018;52(3):296–306.
 22. Dokmanovic L, Milosevic G, Peric J, Tosic N, Krstovski N, Janic D, et al. Next generation sequencing as a tool for pharmacogenomic profiling: Nine novel potential genetic markers for targeted therapy in childhood acute lymphoblastic leukemia. *Srp Arh Celok Lek*. 2018;146(7–8):407–11.
 23. Gutierrez-Camino A, Martin-Guerrero I, Lopez-Lopez E, Echebarria-Barona A, Zabalza I, Ruiz I, et al. Lack of association of the CEP72 rs924607 TT genotype with vincristine-related peripheral neuropathy during the early phase of pediatric acute lymphoblastic leukemia treatment in a Spanish population. *Pharmacogenet Genomics*. 2016;26(2):100–2.
 24. Gilchrist LS, Tanner L. The pediatric-modified total neuropathy score: A reliable and valid measure of chemotherapy-induced peripheral neuropathy in children with non-CNS cancers. *Support Care Cancer*. 2013;21(3):847–56.
 25. Lavoie Smith EM, Li L, Chiang C, Thomas K, Hutchinson RJ, Wells EM, et al. Patterns and severity of vincristine-induced peripheral neuropathy in children with acute lymphoblastic leukemia. *J Peripher Nerv Syst*. 2015;20(1):37–46.
 26. Zgheib NK, Ghanem KM, Tamim H, Aridi C, Shahine R, Tarek N, et al. Genetic polymorphisms in candidate genes are not associated with increased vincristine-related peripheral neuropathy in Arab children treated for acute childhood leukemia. *Pharmacogenet Genomics*. 2018;28(8):189–95.
 27. Liu Y, Fernandez CA, Smith C, Yang W, Cheng C, Panetta JC, et al. Genome-Wide Study Links PNPLA3 Variant With Elevated Hepatic Transaminase After Acute Lymphoblastic Leukemia Therapy. *Clin Pharmacol Ther*. 2017;102(1):131–40.
 28. Fernandez CA, Smith C, Yang W, Liu C, Ramsey LB, Karol SE, et al. Genome-Wide Association Study Identifies PNPLA3 I148M Variant Associated with Elevated Transaminase Levels after Induction Therapy in Pediatric ALL Patients. *Blood*. 2015;126(23):3714.
 29. Zečkanović A, Jazbec J, Kavčić M. Centrosomal protein72 rs924607 and vincristine-induced neuropathy in pediatric acute lymphocytic leukemia: meta-analysis. *Futur Sci OA*. 2020;6(7):FSO582.
 30. Diouf B, Evans WE. Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy: Progress Continues. *Clin Pharmacol Ther*. 2019;105(2):315–7.

Фармакогеномика винкрестином индуковане периферне неуропатије код деце са акутном лимфобластном леукемијом у Србији – искуство једног центра

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САЖЕТАК

Увод/Циљ Винкрестин је један од кључних лекова у протоколима лечења дечје акутне лимфобластне леукемије (АЛЛ). Винкрестин доводи до дестабилизације микротубула, чиме се ћелија зауставља у метафази и индукује апоптоза. Такође доводи до деградације аксона и поремећаја аксонског транспорта, узрокујући винкрестином индуковану периферну неуропатију (ВИПН).

Циљ ове студије био је да истражи повезаност пет варијанти у фармакогеноми уметљеним у метаболизам винкрестина код деце оболеле од АЛЛ која су развила ВИПН, у Србији. Такође, циљ нам је био да откријемо кандидате за нове фармакогеномске маркере ВИПН-а у српској популацији.

Метод Детекција варијанти гена *CYP3A5*, *CEP72*, *ACTG1*, *MIR3117* и *MIR4481* изведена је методологијом заснованом на ПЦР-у и секвенцирању. Статистичким методама је испитана њихова асоцијација са ВИПН-ом код 56 педијатријских болесника оболелих од АЛЛ. Урађена је и популациона винкрестин фармакогеномска анализа 17 фармакогена из постојећих података добијених секвенцирањем нове генерације у српској популацији. Подаци о дистрибуцији

фреквенција алела за европско становништво преузети су из јавних база података.

Резултати Током лечења, 17,86% болесника је развило ВИПН. Асоцијативне анализе показале су да ниједна генетичка варијанта није била повезана са ВИПН-ом у нашој студији. Наше популационо фармакогеномско истраживање није открило валидне фармаковаријанте за ВИПН. Наши резултати не препоручују превентивно фармакогенетичко испитивање винкрестина у Србији.

Закључак Потребан је свеобухватнији приступ како би се идентификовао панел гена којим би се могао објаснити развој ВИПН-а после примене винкрестина код педијатријских болесника оболелих од АЛЛ. Боље осмишљене студије асоцијација на нивоу генома (GWAS) и робуснији алати који користе вештачку интелигенцију довели би до дизајнирања панела фармакогена за превентивно тестирање предиспозиције за развој ВИПН-а, доприносећи индивидуализацији и унапређењу терапије деце оболеле од АЛЛ.

Кључне речи: акутна лимфобластна леукемија; фармакогеномика; винкрестин; винкрестином индукована периферна неуропатија