Nazarbayev University Research Week 2018

INFLUENCE OF CYP2C9 AND VKORC1 GENE VARIANTS ON WARFARIN RESPONSE IN KAZAKHSTANI PATIENTS WITH LEFT VENTRICULAR ASSIST DEVICES

A. Akilzhanova[1], S. Rakhimova[1], S. Andosova[2], Inkar Kalkamanova[3], Aruzhan Tokanova[3], Zh. Zhumadilov[1], M. Bekbossynova[2]
[1]Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan,
[2]National Scientific Cardiac Surgery Center, Astana, Kazakhstan
[3] Nazarbayev Intellectual School, Semey, Kazakhstan

INTRODUCTION

Heart failure is a worldwide epidemic affecting approximately 23 million people globally and is a major burden for the healthcare system [1,2]. Despite advances in medical therapy, the disease is progressive and a significant proportion of patients will need advanced heart replacement therapy.

Left ventricular assist devices (LVAD) are an invaluable part of the therapeutic measures for patients suffering from advanced heart failure. When used either as a bridge to transplant, to promote myocardial recovery, or as lifetime use, LVADs have proven to prolong survival and improve quality of life [1-3]. In spite of their success in improving mortality and quality of life, thrombotic and bleeding events remain significant complications [1]. Achieving therapeutic anticoagulation efficiently with warfarin is important to reduce thrombotic and bleeding risks and is influenced by genotype.

Warfarin has a narrow therapeutic index and displays marked person-to-person variation in dose requirement. Functional polymorphisms at candidate genes can therefore offer utility as biomarkers to individualize warfarin treatment.

Aim of the Study

The main objective of this study was to determine frequency of polymorphisms in CYP2C9, VKORC1 in Kazakhstani patients with mechanical circulatory support - LVADs for further genome-guided anticoagulant therapy.

MATERIALS & METHODS

The study is conducted in accordance with the rules and requirements of the Helsinki Declaration. Written informed consent was obtained from all patients included into the study. Research protocol was approved by the ethics committees of National Laboratory Astana, Nazarbayev University and National Research Center for Cardiac Surgery, Astana, Kazakhstan.

Study population

Implantation of LVADs in Kazakhstan is carried out since 2011 in JSC "National Research Center for Cardiac Surgery ", (NRCCS) Astana, Kazakhstan.
All patients were unrelated Kazakhstani individuals treated in NRCCS, Astana, Kazakhstan due to heart failure. In total, 100 study participants (patients with implanted LVADs) were recruited in the study group during 2011-2016. The control group consisted of 95 conditionally healthy individuals, corresponding to the patients group by sex, age and nationality.

DNA isolation and genotyping

Genomic DNA was extracted from 200 µL whole blood using PureLinkTM Genomic DNA Mini Kit (Invitrogen, UK) according to manufacturer's standard protocol. The genomic DNA concentration was measured using NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, USA) and adjusted to 10 ng/µl.
Genotyping of polymorphisms *VKORC1* and *CYP2C9* genes were performed using RT PCR TaqMan Assay on 7900HT Fast Real-Time PCR System (Applied Biosystems, USA). We examined the *VKORC1* 1639 G>A mutation, *CYP2C9*2* (430 C>T; cytosine to thymine) and the *CYP2C9*3* (1075 A>C; adenine to cytosine) mutations.

Data on the INR level achieved and the most representative warfarin dosage of the patients at discharge or before in-hospital MCS recovery, heart transplant, or Death were gathered.
Data on the bleeding events for up to 18 months after MCS implant or before in-hospital MCS recovery, heart transplant, or death were evaluated.

Patients were divided into two groups for analysis: patients with abnormal warfarin polymorphisms and patients without any polymorphisms. Mean warfarin dosage, mean reached INR, and the rate of bleeding events per patient-year were compared in these two groups.
Demographic data on age, gender, height, weight, ethnicity, obesity, diabetes, hypertension, cardiomyopathy type, and MCS type were collected. We also examined any association between the mutation presence and the numerical variables of age, height, weight, and body mass index (BMI). Furthermore, multivariable analysis was performed to identify any significant predictors for the presence of any of the analyzed polymorphisms.

Statistical Analysis

Continuous variables were summarized by mean ± standard deviation. Normally distributed continuous variables were compared across the two patient groups by the independent samples t-test. Non normally distributed numerical variables were compared across the two groups by the Wilcoxon rank sum test. Categorical variables were summarized by frequency (%). Categorical variables were compared across groups by the Fisher exact test. *P*-value <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS 21 (SPSS Inc., Chicago, IL).

RESULTS

Demographic and clinical profiles of patients are shown in the Table 1.

Warfarin Polymorphisms Genotype Profile of Patients in the Study with frequency of *VKORC1* 1639 G>A mutation, *CYP2C9*2* (430 C>T; cytosine to thymine) and the *CYP2C9*3* (1075 A>C; adenine to cytosine) mutations are show in **Fig 1.** A total of 32.0% (32/100) of patients with implanted LVADs had at least one polymorphism: *VKORC1* (14.0%), *CYP2C9*2* (15.0%), *CYP2C9*2* and *VKORC1* (3.0%).

Table 1. Patient characteristics.

Variables	Subjects (n=100)
Ethnicity: Kazakh	78%
Russian	16%
Other	6%
Average age, years±SD	52,4±11,5
Sex : Male	93%
Female	7%
Weight, kg (range)	79,7±14,0 (48 till 114)
Height, cm (range)	169,9±6,4 (148 till 183)
BMI, kg/m2	27,6±4,6
Smoking, %	58%
Heart Failure etiology: Ischemic genesis	44 %
Non ischemic	56 %
Ischemic cardiomyopathy	44 %
Dilated cardiomyopathy	42 %
Hypertensive cardiomyopathy	9 %
Valvular cardiomyopathy	4 %
Arterial cardiomyopathy	1 %
Type of implanted LVADs:	
•HeartMateIII (Thoratec Corporation, USA)	46%
•HeartMate II (HMII) (Thoratec Corporation, USA)	35%
•HeartWare HVAD (HeartWare Inc., USA)	19%
NYHA class: IIIA+ IIIB	70%
IV	28%
EF, %	21,7 (9 - 41)
LV EDD, mm	70,2 (38 - 88)
Pulse, beats per minute	85,4±17,2
Systolic blood pressure, mm Hg	105,1±15,6
Diastolic blood pressure, mm Hg	87,2±10,9
INR	2.11 ± 0.39 (1.48-3.00)

Anticoagulation therapy is required for patients with implanted LVADs to avoid thrombotic complications. All patients received standard anticoagulation therapy before and after implantation that included adjusted doses of warfarin to achieve the target INR (in most cases, the target INR value is 2.0-2.5 for HMII and HMIII devices, 3.0-3.5 for HeartWare HVAD), and antiplatelet therapy with aspirin. This therapy is adjusted individually for each patient to reduce the risk of ischemic stroke and bleeding.

According to the study protocol, patients are observed within 6-18 months after the implantation of LVAD.

Cases of active bleeding after implantation of the LVAD were observed in only 14% (18 cases) of the total number of subjects. In the first month after implantation, there were only 5 cases of bleeding: 2 cases in the pleural cavity, 2 cases of gastrointestinal bleeding and 1 nosebleed. During the next 6 months after implantation of the device, 6 cases of active bleeding also occurred in 4 patients, including 4 cases of bleeding from the gastrointestinal tract, one case of gingival hemorrhage, and one – nasal bleeding. After 12 months after the operation, two cases of bleeding from the digestive tract were reported in two patients. After 18 months of mechanical support of the left ventricle, 4 new cases of bleeding were observed, 1 case of repeated bleeding, all of them, 3 cases - from the gastrointestinal tract, 1 – nasal bleeding, 1 - rectal bleeding. During the follow-up period, 12% of patients had a thrombosis of the pump (Table 2).

Table 2. Bleeding and thrombosis complications in patients with LVADs implantation during 18 month follow-up

Complication	Number of events	Number of patients	%
Bleeding	18	14	14
Gastro-intestinal	11	9	9
Nasal	3	3	3
Pleural	2	2	2
Other localization	2	2	2
Pomp thrombosis	12	12	12

Frequency of CYP2C9 and VKORC1 Genotype polymorphisms , %

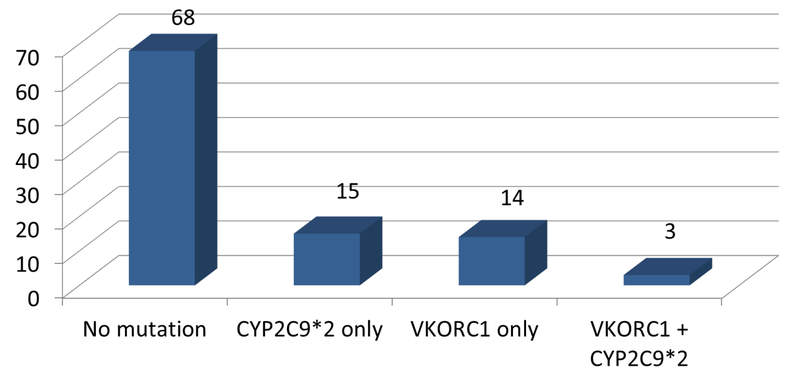

Fig 1. Warfarin Polymorphism Genotype Profile of Patients in the Study

Table 3 shows the differences in INR, warfarin dosage, and bleeding events between the groups with and without one or more polymorphisms (mutations or abnormal warfarin genotypes).

When compared with patients without genotype mutation, patients with mutations in the *VKORC1* and *CYP2C9* genes required a lower mean warfarin daily dosage to reach the same mean INR levels at discharge or before in-hospital mechanical support recovery, cardiac transplantation, or death. Patients in both groups had similar bleeding event rates for up to 18 months or before mechanical support recovery, cardiac transplantation, or death.

Table 3. Clinical Outcomes of Patients in the Mutant (Abnormal Genotype) and Wild-Type (Normal Genotype) Groups

	Genotype		
Variable	Mutant Type (Abnormal) N=32	Wild Type (Normal) N=68	P-value
Warfarin daily dosage, mg	3.2±1.5	5.5±3.7	0.015
INR	2.2±0.67	2.19±0.69	0.96
Bleeding, number of patients,%	5(15.6)	9(13.2)	0.99
Bleeding event rate (event/patient-year)	6.13(4.91-7.55)	8.02(5.93-9.7)	0.13

At discharge or before composite end point, patients with any polymorphism received a lower mean warfarin dosage than patients having no polymorphism (3.2±1.5 *vs*. 5.5±3.7 mg, $p = 0.015$) and achieved a similar mean INR (2.20±0.67 *vs*. 2.19±0.69, $p = 0.96$). There was no significant difference in bleeding rates within 6-18 months or before composite end point (6.13 *vs*. 8.02 events/patient-year, $p = 0.13$).

No association between the presence of a polymorphism in the *VKORC1* and *CYP2C9* genes and age, height, weight, or BMI in LVAD patients were observed.

In multivariable analysis, there were no significant associations etween polymorphism presence and any of the potential predictors including age, BMI, obesity, gender, ethnicity, cardiomyopathy type, or MCS type (data not shown; $p > 0.05$ for all).

CONCLUSIONS

One or more polymorphisms for *VKORC1* or *CYP2C9* (associated with warfarin sensitivity) were found in 32.0% of MCS/LVAD patients.

By using a warfarin genotype-guided approach, LVAD patients with polymorphisms received a lower warfarin dosage to achieve a similar INR, with similar bleeding rates, in comparison with no polymorphisms.

A warfarin genotype-guided approach avoided excessive anticoagulation and its attendant bleeding risks.

REFERENCES

1. Fang JC. Rise of the machines–left ventricular assist devices as permanent therapy for advanced heart failure. N Engl J Med 2009; 361: 2282–5.
2. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, et al. Heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation 2010; 121: e46–215.
3. Miller LW, Guglin M. Patient selection for ventricular assist devices: a moving target. J Am Coll Cardiol 2013; 61: 1209–21.

Ainur Akilzhanova, MD, PhD, DMSci, Assoc.Prof.,
Center for Life Sciences, National Laboratory Astana, Nazarbayev University,
53 Kabanbay Batyr ave., 010000, Astana, Republic of Kazakhstan, Tel: +7(717)2706501, e-mail: akilzhanova@nu.edu.kz, akilzhanainur@gmail.com, http://www.nu.edu.kz