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Pharmacogenetic algorithm for predicting daily dose of warfarin in Caucasian patients of Czech origin

<https://doi.org/10.1515/dmdi-2020-0171>

Received October 27, 2020; accepted November 21, 2020;
published online ■■■

Abstract

Objectives: Warfarin use is limited by a low therapeutic index and significant interindividual variability of the daily dose. The most important factor predicting daily warfarin dose is individual genotype, polymorphisms of genes CYP2C9 (warfarin metabolism) and VKORC1 (sensitivity for warfarin). Algorithms using clinical and genetic variables could predict the daily dose before the initiation of therapy. The aim of this study was to develop and validate an algorithm for the prediction of warfarin daily dose in Czech patients.

Methods: Detailed clinical data of patients with known and stable warfarin daily dose were collected. All patients were genotyped for polymorphisms in genes CYP2C9 and VKORC1.

Results: Included patients were divided into derivation (n=175) and validation (n=223) cohorts. The final algorithm includes the following variables: Age, height, weight, treatment with amiodarone and presence of variant alleles of genes CYP2C9 and VKORC1. The adjusted coefficient of

determination is 72.4% in the derivation and 62.3% in the validation cohort ($p < 0.001$).

Conclusions: Our validated algorithm for warfarin daily dose prediction in our Czech cohort had higher precision than other currently published algorithms. Pharmacogenetics of warfarin has the potential in the clinical practice in specialized centers.

Keywords: CYP2C9; daily dose prediction algorithm; pharmacogenetics; VKORC1; warfarin.

Introduction

Despite the introduction of direct oral anticoagulants, warfarin remains one of the most widely used anticoagulant drugs worldwide with clinical efficacy in the prevention and treatment of thromboembolic complications. Owing to healthcare payers' recommendations warfarin remains the first-choice drug in the Czech Republic. Its use is limited by a low therapeutic index and significant interindividual variability of the daily dose. A major factor predicting daily warfarin dose is individual genotype, polymorphisms of genes CYP2C9 (warfarin metabolism) and VKORC1 (sensitivity for warfarin) [1].

CYP2C9 gene encodes a member of the cytochrome P450 superfamily of enzymes, 2C9 isozyme, responsible for warfarin metabolism (pharmacokinetics). Allele *1 in homozygous form *1/*1 is most prevalent in our population (67.6% of the population) [2]. *1/*1 homozygotes are extensive metabolizers of warfarin and require a standard dose of warfarin. Carriers of variant alleles *2 (20.6% of the population) and *3 (14.3% of the population) are poor metabolizers and require a significantly lower dose of warfarin [2–4].

A second major gene for warfarin is VKORC1, which encodes subunit 1 of vitamin K epoxide reductase complex (pharmacological target of warfarin) and so determines individual “sensitivity” for warfarin (pharmacodynamics). About 44.7% of Czech population carry A/B haplotype and

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have standard sensitivity for warfarin [2]. The variant allele in homozygous form (A/A haplotype, high sensitivity for warfarin) is present in 14% of Czech population and is significantly associated with a lower dose of warfarin [2, 5–8], and less significantly with shorter time to reach therapeutic range and higher probability of overdose [9, 10].

Patient's total number of variant alleles is a possible scoring system for clinical practice. About 3 or 4 variant alleles imply increased risk of adverse effects. Carriers of 3 variant alleles have been reported to have 4.34 times higher risk of major bleeding ($p < 0.001$) [11]. Healthcare payers recognized this fact in the Czech Republic. The biggest Czech insurance company (Všeobecná zdravotní pojišťovna) accepts pharmacogenetics as an indication for novel oral anticoagulants in case of high-risk patients—carriers of 3 or 4 variant *CYP2C9* and *VKORC1* alleles.

Apart from estimating the risk of bleeding complications, pharmacogenetics can be used to estimate daily warfarin dose prior to treatment initiation. This dose can be predicted by a pharmacogenetic algorithm, i.e., dosing equation identified through multivariate regression analysis. Patient's clinical and genetical data are put in to compute the estimated daily dose. Many such algorithms have been published and use various input variables [1]. The accuracy of the algorithms is reported by the coefficient of determination (R^2), which ranges in between 0% (absolute error) to 100% (precise estimate). R^2 of dosing algorithms published so far was between 31 and 62% [1]. One of the significant factors influencing predictive value is the ethnic origin of patients in the derivation cohort, which should be similar to the target population.

The aim of the study was to develop a novel pharmacogenetic dosing algorithm based on clinical data and genotype of a cohort of Czech patients treated with warfarin (the derivation cohort) and subsequently assess its precision on an independent validation cohort of patients and compare it to the previously published dosing algorithms.

Materials and Methods

Patient eligibility

Patients for both derivation and validation cohorts were recruited from following neurological clinics: Department of Neurology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Department of Neurology in Kladno Regional Hospital, Department of Neurology in Chomutov Regional Hospital

and Hospital Na Homolce. All the patients who participated in the study provided written informed consent and the study protocol was approved by the local Ethics Committee.

More than 150 items of clinical data were collected from each patient, e.g., age, height, weight, indication of warfarin treatment, comorbid medical conditions such as elevated systolic blood pressure, diabetes mellitus, oncology disorders, renal and hepatic disease etc., concomitant medication (prescription and over-the-counter drugs as well as vitamins and herbal products), and comprehensive data concerning warfarin treatment (the daily maintenance dose, number of dose adjustments, INR measurements). Clinical data were collected from inpatient and outpatient medical records and directly from patients during regular planned visits and by structured telephone interview.

Only patients with stable control of anticoagulation and for whom complete clinical data were available were included in the study. A stable patient was defined as one who was treated with warfarin for a minimum period of 90 days and with the last two INR measurements within the therapeutic range (this range was defined from 1.8 to 3.2 to allow for measurement error) taken at least 30 days apart on the same daily dose of warfarin and with no dose adjustment in between. To achieve higher accuracy of the algorithm we set a minimum age limit of 50 years, as we expected a decrease in a daily dose in older patients. This restriction can consequently increase precision for younger patients. More carriers of 4 variant alleles than based on population frequency were included in the study to achieve higher predictive value for this subgroup of patients most likely to suffer from adverse events.

A second unrelated cohort of patients (validation cohort) was used to assess the novel dosing algorithm. Apart from no age limit applicable, the inclusion criteria were identical to the derivation cohort. The sample size was estimated as 150 patients as there is no standardized test for sample size calculation for algorithm development.

More important than the total number of patients is the accuracy of clinical data (exact weight, a stable daily dose of warfarin, etc.). Therefore, each patient was carefully selected, and data validity was assessed—by a secondary check of database data, directly by a physician during their regular planned visits, by examining the inpatient and outpatient records and by structured telephone interview. Furthermore, only patients of Czech (Caucasian) origin (self-declared) were included. We applied no other selection criteria.

Genotyping

Five milliliters of peripheral blood was obtained during a regularly scheduled blood draw. Genomic DNA was isolated from whole blood with Puregene Blood Kit (Qiagen Inc., Chatsworth, CA, USA). Genotyping was performed using the high-resolution DNA melting technique. The Genotyping Master Mix (Cat. No. HRLS-ASY-0006, Idaho Technology Inc., Utah, USA) according to the manufacturer's protocol and primer/probe reagents designed for detection of alleles * 2 and * 3 of the gene *CYP2C9* and single nucleotide polymorphism 1639-A>G of gene *VKORC1* (Cat. No. HRLS-ASY-007, HRLS-ASY-0010, HRLS-ASY-0011, Idaho Technology Inc., Utah, USA) were used for polymerase chain reaction in a PCR cycler MultiGene (Labnet International, Inc., Edison, NJ, USA).

Statistical analysis

Hierarchical multiple regression analysis on the derivation cohort data was performed to develop a new warfarin dosing algorithm. Only statistically significant variables with a p-value ≤ 0.1 on univariate testing and which met the assumption of linearity to the dependent variable (the actually used daily dose of warfarin) were added to the final model.

Following input variables were included in the statistical analysis:

- Genetic characteristics—*CYP2C9* *2,*3 polymorphisms, *VKORC1* (A/A, A/B, B/B) haplotypes
- Baseline patient characteristics—sex, age, weight and height
- Medical history data—the indication of warfarin treatment, comorbid conditions (ischemic stroke, ischemic heart disease, diabetes mellitus, elevated systolic blood pressure, renal and hepatic disease, deep vein thrombosis or pulmonary embolism, smoking and daily alcohol consumption)
- Concomitant medication influencing daily warfarin dose (amiodarone, statins—simvastatin, atorvastatin and rosuvastatin in particular, carbamazepine, phenytoin, sartans—losartan, irbesartan, valsartan, telmisartan, spironolactone, sulodexide, furosemide, sulfonylurea derivatives, hydrochlorothiazide, corticosteroids—prednisone).

Independence of residuals in the final model was assessed by a Durbin-Watson statistic. Casewise diagnostics was performed to identify possible outliers, i.e., cases with standardized residuals of more than three standard deviations (SD) away from the mean. The residuals of the model were tested for the assumption of normality and homoscedasticity. Statistical analysis was done using IBM SPSS Statistics 18.0 (SPSS, Inc., Chicago, Illinois, USA).

The algorithm was assessed on the validation cohort of patients and compared to already published algorithms using methods published by our team [2]. Finally, a Bland-Altman plot was created in GraphPad Prism v. 6.0 (GraphPad Software, Inc., USA).

Results

Clinical and genetic characteristics of patients in the derivation and validation cohorts

One hundred seventy-five patients were included in the derivation cohort, 223 patients were included in the independent validation cohort. All patients included in the study met the requirement of stable control of anticoagulation, the median of warfarin treatment was 26 months, the first quartile was 15 months.

The patients' demographics, clinical data and genotype relevant for algorithm development are shown in Table 1. There was a significant difference in age ($p < 0.001$) and there was a higher frequency of women in the derivation cohort, other characteristics were not statically significantly different.

Warfarin dosing algorithm

We identified following statistically significant variables which reached a p-value ≤ 0.1 on univariate testing and which met the assumption of linearity to the dependent variable (actually used daily dose of warfarin): number of *2 and *3 *CYP2C9* polymorphisms, *VKORC1* A/A, A/B and B/B haplotypes, age, weight, height, use of amiodarone and use of statin.

Table 1: Relevant clinical characteristic and genotype of the derivation and validation cohorts compared using the Mann-Whitney U test for continuous variables and χ^2 test for categorical variables.

Variable	Derivation Cohort	Validation Cohort	P
Number of patients	175	223	–
Mean age $\pm$ SD (range), years	68.3 $\pm$ 9.61 (50–89)	60.17 $\pm$ 18.07 (13–89)	<0.001*
Mean body weight $\pm$ SD (range), kg	83.0 $\pm$ 15.61 (42–125)	83.7 $\pm$ 19.09 (45–190)	0.212
Mean body height $\pm$ SD (range), cm	172.4 $\pm$ 9.71 (150–200)	171.4 $\pm$ 9.3 (148–198)	0.252
Women	69 (39.4%)	118 (52.9%)	0.007*
Mean daily dose $\pm$ SD (range), mg	4.50 $\pm$ 2.08 (1.0–12.8)	5.19 $\pm$ 2.84 (1.13–18)	0.058
Use of amiodarone	35 (20.0%)	28 (12.6%)	0.055
Use of statin (simvastatin, atorvastatin, losuvastatin, fluvastatin)	63 (36.0%)	96 (43.0%)	0.154
1 variant allele <i>CYP2C9</i> *2	38 (21.7%)	33 (14.8%)	0.082
2 variant alleles <i>CYP2C9</i> *2	3 (1.7%)	1 (0.4%)	0.324
1 variant allele <i>CYP2C9</i> *3	24 (13.7%)	26 (11.7%)	0.546
2 variant alleles <i>CYP2C9</i> *3	2 (1.1%)	2 (0.9%)	1.0
<i>VKORC1</i> A/A	27 (15.4%)	29 (13.0%)	0.626
<i>VKORC1</i> A/B	81 (46.3%)	99 (44.4%)	0.761
<i>VKORC1</i> B/B	67 (38.3%)	95 (42.6%)	0.412

Table 2: Regression coefficients for individual variables of the final model and their statistical significance.

Variable	Regression Coefficient	SD, Standard deviation	Statistical Significance, P	95% Confidence interval
Constant	2.049	0.417	<0.001	1.225 to 2.872
Age	-0.016	0.002	<0.001	-0.020 to -0.012
Height	0.007	0.002	0.004	0.002 to 0.012
Weight	0.004	0.002	0.010	0.001 to 0.007
Allele 2	-0.227	0.042	<0.001	-0.309 to -0.145
Allele 3	-0.296	0.050	<0.001	-0.394 to -0.199
VKORC	-0.340	0.029	<0.001	-0.397 to 0.284
Amiodarone	-0.397	0.048	<0.001	-0.491 to -0.303

Following variables were included in the final model: age, weight, height, *CYP2C9* and *VKORC1* genotype, and use of amiodarone, which have a statistically significant predictive value for warfarin daily dose $F(7, 167)=66.120$, $p<0.001$ (shown in Table 2).

The final algorithm is summarized as follows:

$\sqrt{\text{Daily dose, mg}} = (2.049 - (0.016 * [\text{age, years}]) + (0.007 * [\text{height, cm}]) + (0.004 * [\text{weight, kg}]) - (0.227 * [\text{CYP2C9} * 2, \text{ number of alleles } 0-2]) - (0.296 * [\text{CYP2C9} * 3, \text{ number of alleles } 0-2]) - (0.340 * [\text{VKORC}, A/A=0, A/B=1, B/B=2]) - (0.397 * [\text{use of amiodarone, no}=0, \text{ yes}=1])$.

Statistical significance of individual variables is shown in Table 2. The final algorithm reaches high predictive value with the coefficient of determination $R^2=73.5\%$ (the adjusted R^2 is 72.4%). There was the independence of residuals, as assessed by a Durbin-Watson statistic of 2.016 (the test statistic ranges in between 0 and 4, a value of 2 indicates that the residuals are not autocorrelated and there is excellent independence of observations). There were no studentized deleted residuals greater than ± 3 standard deviations. The residuals of the model met the assumption of normality and homoscedasticity.

Validation of the algorithm

Daily warfarin dose predicted by the final algorithm compared to the actual warfarin dose reaches the coefficient of determination $R^2=84.8\%$ ($p<0.001$) in the

derivation cohort (cohort used for algorithm development). However, there is bias in this predictive value due to the way the algorithm was developed. Therefore, the algorithm was assessed on the validation cohort with the coefficient of determination $R^2=62.3\%$ ($p<0.001$). Mean absolute error of prediction was 1.57 ± 1.59 mg with a median of 1.12 mg.

The second compared parameter was if the estimated dose was within the range of $\pm 20\%$ used daily dose. The mean absolute percentage error was 35.5% with a median 22.4% (SD 41.06). 48.6% of patients were correctly predicted within the range $\pm 20\%$ used daily dose. Eighty-eight patients (39.3%) out of 223 would be overdosed, i.e., predicted dose was higher than the actually used dose. However, only in 29.5% of the overdosed patients, there was an error of more than 2 mg and all of these patients used daily dose of warfarin higher than 5 mg.

About 126 (56.5%) patients would be underdosed (a predicted dose lower than the actually used dose) in average by 1.41 ± 1.12 mg. In 46.8% of underdosed patients, the error was less than 2 mg.

The algorithm was also assessed on patient's subgroups with the following results. The model is less precise for patients under 50 years of age ($n=61$), with $R^2=38.8\%$. On the contrary, the prediction was sufficiently accurate for patients over 80 years of age ($n=20$)—52.7%. The coefficient of determination was 57.7% for patients with excess body weight (>100 kg, $n=34$) and due to the small number effect even 97.7% for patients with body weight under 50 kg ($n=3$) (see Figure 1).

$\sqrt{\text{Daily dose, mg}} = (2.049 - (0.016 * [\text{age, years}]) + (0.007 * [\text{height, cm}]) + (0.004 * [\text{weight, kg}]) - (0.227 * [\text{CYP2C9} * 2, \text{ number of alleles } 0-2]) - (0.296 * [\text{CYP2C9} * 3, \text{ number of alleles } 0-2]) - (0.340 * [\text{VKORC}, A/A = 0, A/B = 1, B/B = 2]) - (0.397 * [\text{use of amiodarone, no} = 0, \text{ yes} = 1])$

Figure 1: The final algorithm to estimate daily dose of warfarin. Variables in square brackets are to be substituted for by values valid for individual patients.

Discussion

Our proposed pharmacogenetic dosing algorithm was validated on the validation cohort with the coefficient of determination $R^2=62.3\%$ ($p<0.001$). This means excellent precision compared to published warfarin dosing algorithms, which use only on the data available before treatment initiation. Similar precision was reported only for the algorithms by Tham in East Asian population—60.2% [12], by Miao in Chinese population—62.8% [13] and by Wadelius in Swedish population—59% [14].

When compared to other published algorithms used to predict daily warfarin dose in our validation cohort—by Anderson with $R^2=50.1\%$ ($p<0.001$) [15] and by Sconce 55.3% ($p<0.001$) [5], our algorithm showed significantly statistically higher precision in Czech patients. Moreover, both algorithms were complemented with adjustment for target INR and correction for amiodarone treatment. If we compared our algorithm to those published algorithms without this correction, we would get probably even more precise results.

Figure 2 depicts Bland-Altman plots comparing daily warfarin dose predicted by our algorithm compared to already published algorithms. Bland-Altman plot expressed numerically would include estimated bias (the mean of the differences) and 95% limits of agreement with the following results for our validation cohort:

- Our dosing algorithm x algorithm by Sconce:
Bias -0.2905 , SD 1.325 , 95% limits of agreement -2.888 to 2.307
- Our dosing algorithm x algorithm by Anderson:
Bias 0.1370 , SD 0.7670 , 95% limits of agreement -1.248 to 1.522

Our proposed dosing algorithm is quite comparable to previously published algorithms in patients of our validation cohort. It is in better agreement with the algorithm by Sconce, which showed higher precision in our patients.

An advantage of our proposed algorithm is that, if the daily dose is not predicted accurately, the patients are more likely to be underdosed than overdosed: 56.5 and 39.3% of patients, respectively. Moreover, clinically potentially relevant overdose (error of more than 2 mg) occurred only in 29.5% of overdosed patients, i.e., in 11.6% patients of the validation cohort. Underdose was not only more frequent but also more significant as for the error in the predicted dose. An error of more than 2 mg was recorded in 46.8% of the underdosed patients, i.e., 26.4%

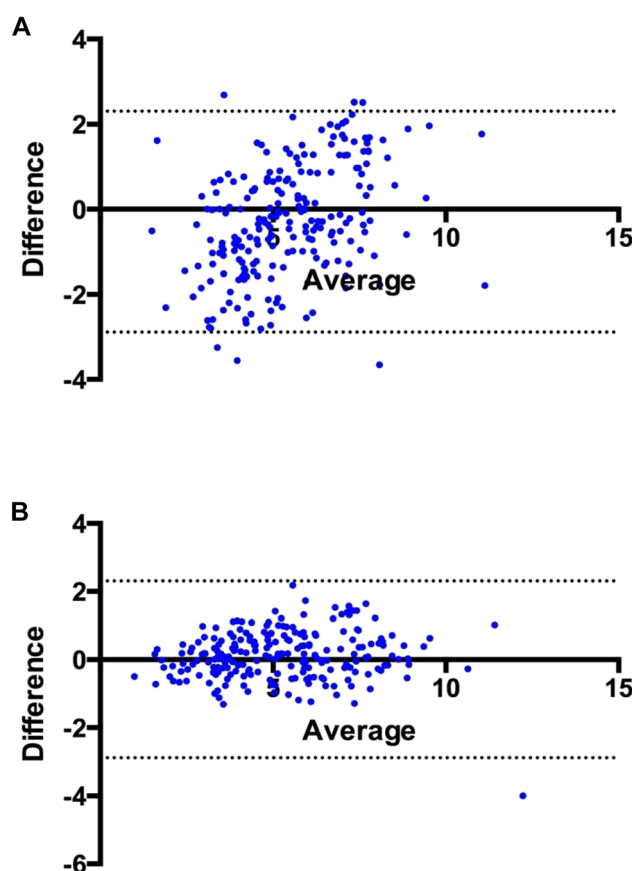


Figure 2: Bland-Altman plot evaluating the agreement of estimated warfarin daily dose predicted by our proposed dosing algorithm compared to the algorithm (A) By Anderson (B) by Sconce.

The prediction was sufficiently accurate to be used in clinical practice for patients over 80 years of age (52.7%), for patients with body weight >100 kg (57.7%) and with body <50 kg (97.7%).

A limitation of our proposed algorithm is its lower accuracy in patients under 50 years of age ($R^2=38.8\%$). When assessing overall predictive value, it has to be taken into account that there was a significant difference in age between the derivation and validation cohort, caused by setting a minimum age limit of 50 years for the derivation cohort; patients in the validation cohort were significantly younger (Table 1). However, other published algorithms developed on derivation cohorts with a higher mean age show analogically low precision.

This inaccuracy is due to different explanatory variables, or their coefficients, in younger, especially pediatric patients. There is a significantly higher influence of age, body weight and height in younger patients than in the older population. Pharmacogenetic data are less clinically

significant in younger patients and therefore the predicted doses for patients under 50 years of age should be viewed with caution. For example, the influence of age ranges from 29.8 to 40% in published pediatric cohorts [16], which is significantly higher than in older adult cohorts (approximately 10% [4]). The only way to improve predictive value for younger patients is to develop a special dosing algorithm for this subgroup of patients [16].

Conclusions

Our validated algorithm for warfarin daily dose prediction shows high precision, fully comparable to the most precise previously published algorithms. An advantage for clinical practice is a more frequent prediction of lower than actually needed daily warfarin dose, i.e., more likely underdose than overdose. However, its main advantage is development and subsequent validation in Czech patients. Pharmacogenetics of warfarin is a strategy with a potential to be used to estimate daily warfarin dose especially for patients at high risk of bleeding complications such as patients after recent ischemic stroke.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

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