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Pharmacodynamic Modeling of Warfarin Dosing Algorithm for Cardiovascular Patients in Indonesia: A Tailored Method to Anticoagulation Therapy

Norisca Aliza Putriana[1], Irma Rahayu Latarissa[2], Taofik Rusdiana[1], Tina Rostinawati[3], Mohammad Rizki Akbar[4]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia; [2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia; [3]Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia; [4]Department of Cardiovascular, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia

Correspondence: Norisca Aliza Putriana, Department of Pharmaceuticals and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM. 21, Jatinangor, 45363, West Java, Indonesia, Email norisca@unpad.ac.id

Purpose: Warfarin is an anticoagulant drug widely used for treating thromboembolism-related conditions. The main challenge with this drug is the high variability in patients response, which is influenced by both clinical, non-clinical, and genetic factors, such as *VKORC1, CYP2C9*, and *CYP4F2*. Therefore, this research aimed to evaluate the impact of clinical and genetic factors on warfarin dose adjustment and to develop a dosing algorithm for patients with cardiovascular disease.

Patients and Methods: A total of 77 research subjects were selected using consecutive sampling based on the inclusion criteria of cardiac outpatients on warfarin for ≥3 months with PT-INR data, complete medical records, and willingness to participate. Exclusion criteria included vitamin K use and inability to follow up. Patients demographic data and clinical characteristics were collected from medical records. Blood samples were obtained for genetic testing of *CYP4F2 rs2108622* (sequencing). Statistical analyses included both bivariate and multivariate analyses (logistic regression) with a significance level set at <0.05.

Results: Statistical analysis using the Kruskal–Wallis test showed that the CC, CT, and TT genotypes were significantly associated with warfarin dose ($p = 0.02$). Furthermore, the Mann–Whitney test results showed that gender did not have a significant relationship with warfarin dose ($p = 0.16$). The Spearman Rank correlation test showed that age ($p = 0.02$) and BMI ($p = 0.03$) had significant relationships with warfarin dose ($p < 0.05$). However, gender ($p = 0.89$) had no effect, while age ($p = 0.01$), BMI ($p = 0.01$), and genotype ($p = 0.01$) significantly influenced warfarin dose determination.

Conclusion: In conclusion, the combined contribution of age (8.76%), BMI (7.95%), and *CYP4F2* genotype (8.29%) to warfarin dose adjustment was 25%. The linear regression model for predicting warfarin dose was determined to be y = 12.736–0.16*age + 0.55*BMI + 3.55*genotype, where 1 = CC, 2 = CT, and 3 = TT.

Keywords: warfarin dosing, cardiovascular disease, CYP4F2 genotype, anticoagulation therapy, genetic polymorphism

Introduction

Warfarin is a class of anticoagulant drugs that are often used to treat diseases associated with thromboembolism, such as atrial fibrillation, venous thrombosis, and pulmonary thrombosis.[1,2] The main problem with the use of warfarin is that the variation in response between patients is very high.[3] This causes difficulty in determining the initial dose of each patients appropriately, which will then result in the occurrence of DRP (drug-related problem) cases in the form of adverse drug reactions.[3–5] The high variation occurs due to the uniqueness of the drugs, which has the characteristics of a narrow therapeutic index. Therefore, underdose condition results in inadequate treatment or complications, while overdose leads

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to bleeding phenomena, ranging from severe instances such as cerebral hemorrhage to minor cases, namely ocular bleeding.[6–9]

During the COVID-19 pandemic, the use of anticoagulants, including warfarin, gained significant attention due to the increased risk of thromboembolic complications in infected patients.[10–13] This highlights the critical need for precise warfarin dosing, as mismanagement could exacerbate complications related to both thromboembolism and bleeding. A previous study showed that 44% of patients who experienced bleeding had an INR value >3.0, whereas 48% of patients with thromboembolic events had an INR value <2.15.[14] These findings highlight the significant risks associated with improper dosing and the need for careful monitoring of INR values in warfarin therapy.

Some of the factors that cause significant variations in response to warfarin use include clinical/demographic (age, weight, gender, body surface area, disease), non-clinical, and genetic factors (*VKORC1, CYP2C9, CYP4F2*).[15,16] Previous research has shown that genetic factors *VKORC1* and *CYP2C9* significantly influence variations in the pharmacokinetic and pharmacodynamic responses of warfarin.[17] Patients carrying the homomutant *VKORC1* gene type carrier (AA) show a low warfarin dose requirement, while the *VKORC1* gene type (GG) tends to require a higher dose. Meanwhile, patients with homomutant (*3/*3) type carriers of *CYP2C9* are at great risk of side effects in the form of bleeding. This condition necessitates the administration of warfarin at low doses. *CYP2C9* wildtype (*1/*1) tends to require higher doses and risk disease complications when given standard doses.[18]

In recent research, another SNPs that could potentially influence warfarin therapy was found, namely *CYP4F2 rs2108622*. *CYP4F2* catalyzes the conversion of vitamin K to its inactive metabolite, hydroxyvitamin K.[19] The *rs2108622* V433M variant results from a C > T nucleotide substitution, where the T allele replaces valine with methionine at position 433, reducing catalytic activity and potentially affecting blood clotting and warfarin response.[17]

A dosing algorithm model was needed to determine the appropriate initial and maintenance doses for patients receiving warfarin therapy. Several countries have developed algorithmic models to determine warfarin doses that are influenced by clinical, non-clinical, and genetic factors. Some of these models include Japan (Dose = 2.263 + 4.248 x (*VKORC1* G/G) + 1.067 x (*VKOCR1* A/G) − 2.416 x (*CYP2C9**3/*3) − 0.864 (x*CYP2C9**1/*3) + 1.308 x *BSA* + 0.025 x age), in China (Dose = 0.727–0.007 x age + 0.384 x *BSA* + 0.403 x (*VKORC1* G/A) + 0.554 x (*VKORC1* G/G) − 0.482 x (*CYP2C9**1/*3) − 1.583 x (*CYP2C9**3/*3), in Italy (Dose = 7.39764–0.02734 x age + 1.06287 x *BSA* − 1.04468 x *VKORC1* A/G − 2.12117 x *VKORC1*), and USA (Dose = 3.52–0.006 x age + 0.38 x BSA − 0.15 x *hypertension* − 0.23 x (*CYP2C9**1/*3 or *3/*3) − 0.24 x (*VKORC1* A/G) − 0.48 x (*VKORC1*).[20–22] In Indonesia, there is still no development of this warfarin dosing algorithm model. Therefore, this research aimed to obtain a model of warfarin dosing algorithm or pattern according to the condition of each patient. The results can be applied as a guide in warfarin therapy in cardiac hospitals or clinics where cardiologists treat patients using warfarin.

Materials and Methods

Ethics Statement

This research complies with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the West Java Health Ethics Commission-Faculty of Medicine, Universitas Padjadjaran with registration number 1342/UN6.KEP/EC/2019.

Subjects

The inclusion criteria were outpatients of the cardiac clinic who had been on warfarin therapy for ≥ 3 months, had Prothrombin Time-International Normalized Ratio (PT-INR) laboratory data available, had complete medical records, made routine medical visits, and were willing to participate. Similarly, the exclusion criteria were patients who took supplements containing vitamin K, and those who could not be followed up due to death, relocation of treatment, or inability to be contacted.

The sample size required for this study was calculated using the Lemeshow formula based on the allele prevalence:

$$n(1+x)^n = \frac{NPrev(1-Prev)}{(n-1)\frac{d^2}{\left(Z1-\frac{\alpha}{2}\right)^n} + Prev(1-Prev)}$$

Explanation of variables:

n: required sample size

d: margin of error (5%)

N: population size

Prev: prevalence of the CYP4F2 polymorphism (31.45% in the Asian population, as reported by Singh et al, 2011)[17]

Z: confidence level (95%, corresponding to 1.96)

Given that the population of warfarin therapy patients at Hasan Sadikin Hospital, Bandung, was 100, and the polymorphism prevalence (C > T) was 31.45%, the calculation is as follows:

$$n(1+x)^n = \frac{100x0.3145(1-0.3145)}{(100-1)x\frac{0.05^2}{1.96^2} + 0.3145(1-0.3145)} = 76.99 = 77sample$$

All patients provided informed consent, then clinical characteristics, medical history, medications used, and daily warfarin doses were recorded. Clinical data were collected by reviewing medical records and direct inquiry during regular scheduled clinic visits. The clinical data included age, height, weight, gender, target INR, concomitant diseases, combined medications, and warfarin dosage.

Blood Sampling

A 3 mL blood sample was collected into marked EDTA tubes and stored at −20°C. The design of gene-specific primers for *CYP4F2 rs2108622* was carried out by downloading the gene sequence from the National Center for Biotechnology Information (NCBI). After obtaining the sequence, the nitrogenous base sequence was input into the Primer-BLAST tool on the NCBI website (www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers were then verified using the online OligoCalc software (http://biotools.nubic.northwestern.edu/OligoCalc.html). The primers are shown in Table 1, respectively.

Deoxyribonucleic Acid (DNA) Extraction and Genotyping

A total of 200 µL of blood was placed in a 1.5 mL Eppendorf tube and 20 µL of proteinase K and 20 µL of Ribonuclease (RNAse) A solution were added. The mixture was homogenized by vortexing, then 200 µL of lysis solution C was added to the Eppendorf tube, and the tube was vortexed again for 15 seconds. The mixture was then incubated for 10 minutes at 55°C. After incubation, 200 µL of 95% ethanol was added to the lysate, and the mixture was homogenized by vortexing for 10 seconds.

DNA purification was performed using GenElute™ miniprep binding columns. The lysates, previously mixed with 95% ethanol, were transferred into the columns and centrifuged at 6,500 x g for one minute. The liquid in the collection tubes (2.0 mL) was discarded and replaced. The next step in the DNA purification process was the washing stage, using a wash solution concentrate that had been diluted with 95% ethanol. The DNA extraction process was concluded with the elution stage, where 100 µL of elution solution was added to the column and centrifuged at 6,500 x g for one minute, and the process was repeated twice.

The Polymerase Chain Reaction (PCR) process consists of three stages, namely denaturation, annealing, and extension. Several temperature variations were used to determine the optimal primer annealing temperature, including 55.4°C, 56.4°C, 57.4°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63.4°C, and 64.4°C. The total reaction volume was 25 µL,

Table 1 Primer

Gen *CYP4F2*	Primer	Fragment Size
Rs2108622	Forward 5' TACTCCTGATCAAAACCCTGCC 3'	170 pb
	Reverse 5'CTTCTCCTGACTGCTCCCTT 3'	

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comprising 2 µL of DNA template, 1 µL of forward primer, 1 µL of reverse primer, 12.5 µL of PCR Master Mix, and 8.5 µL of nuclease-free water. The PCR product was then electrophoresed on a 2% agarose gel at 80 volts for 90 minutes. The electrophoresis results were visualized under UV light at 312 nm using a fluorescence scanner. The PCR products were then sent to Humanizing Genomics Macrogen (https://www.macrogen.com/en/main/index.php), Korea, for sequencing. Sequencing was performed using the Sanger method, which relied on DNA synthesis with chain termination.

Statistical Analysis

The characteristics of the data were assessed to determine the normality using the D'Agostino or Kolmogorov–Smirnov tests. Based on the results, appropriate statistical test methods were applied. For normally distributed data, ANOVA or Student's *t*-test was used for analysis, at a significance level of $\alpha = 0.05$. Otherwise, the Kruskal–Wallis or Mann–Whitney *U*-test was applied.

Univariate analysis was conducted for descriptive analysis to determine the characteristics of each research variable, presented as number and percentage (n, %). Bivariate analysis was conducted to identify variables that could be included in the multivariate model, with a p-value < 0.05. Furthermore, the multivariate regression analysis (logistic regression) was used to examine the correlation and develop warfarin dosing model, considering both clinical and non-clinical factors, with a p-value < 0.05.

Results

A total of 77 patients participated in this research from March to December 2021. Demographic data and clinical characteristics of patients were obtained by reviewing medical records. Table 2 shows the description of patients demographic characteristics.

Table 2 Baseline Demographic, Clinical Characteristic and Mean INR Value

Variables	Value	Mean INR Value
Median age (range) (year)	54 (28–80)	–
Male/female N (%)	37/40 (48/52)	2.57/2.96
Mean BMI (range) (kg/m^2)	23.63 (17–35)	–
Mean warfarin dose (range) (mg/week)	20.25 (7–46)	–
Mean INR < 2 N (%)	29 (37)	–
Mean INR 2–3 N (%)	41 (53)	–
Mean INR > 3 N (%)	7 (9) • CC: 4 • CT: 3 • TT: 0	–
Primary indication N (%)		
Rheumatic Heart Disease	32 (41.60)	2.04
Atrial Fibrillation	25 (32.50)	2.7
Mitral Valve Prolapse	8 (10.40)	2.43
Coronary Artery Disease	7 (9.10)	2.12
Hypertensive Heart Disease	5 (6.50)	2.3

(*Continued*)

Table 2 (Continued).

Variables	Value	Mean INR Value
Concomitant medication N (%)		
Erythromycin	35 (46.10)	2.31
Spironolactone	15 (19.70)	2.09
Sucralfate	3 (3.90)	2.66
Lansoprazole	11 (14.50)	2.01
Simvastatin	3 (3.90)	2.36
Allopurinol	2 (2.60)	2.71
Diltiazem	1 (1.30)	1.84
Comorbidities		
Hypertension	7 (9.2)	2.26
Diabetes Mellitus	2 (2)	2.47
Turner Syndrome	1 (1)	2.91
Epilepsy	1 (1)	2.57
Hyperthyroidism	2 (2)	2.36
Tuberculosis	1 (1)	2.85
Hyperlipidemia	2 (2)	3.01
Gout	3 (3)	2.07
CY4F2 (*rs2108622*) N (%)		
CC	47 (61)	2.2
CT	27 (35)	2.4
TT	3 (4)	2.7

Abbreviation: BMI, Body Mass Index; INR, International Normalized Ratio.

The average weekly dose based on age, Body Mass Index (BMI), and *CYP4F2* rs 2108622 genotype are shown in Table 3. The results showed that the required dose decreases with increasing age. Specifically, patients aged 70–79 required a weekly dose of 16.17 mg, which is 27.33% lower than the highest average dose for patients aged 30–39, while patients aged 80–89 required a significantly lower dose of 7 mg (3 times smaller than the largest dose).

Bivariate Analysis

The results of the bivariate analysis between patients demographics and genotypes on warfarin dose are shown in Table 4. Variables with a p-value <0.25 in the bivariate analysis are eligible to enter the multivariate model.

The Kruskal–Wallis test on genotype showed a p-value of 0.02 (<0.05), suggesting that the CC, CT, and TT genotypes have a significant association with warfarin dosage. Meanwhile, the Mann–Whitney test on gender had a p-value of 0.16 (>0.05). This result showed that gender does not have a significant relationship with warfarin dosage. However, gender was included in the multivariate analysis ($p < 0.25$) as a confounding factor.

The results of the Spearman Rank correlation analysis for age ($p = 0.02$) and BMI ($p = 0.03$) showed p-values <0.05. This implies that age and BMI have a significant relationship with warfarin dosage. The correlation coefficient values from this analysis were −0.28 for age and 0.25 for BMI. These results suggest that the strength of the relationship

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Table 3 Mean Weekly Doses (in Mg) for Age, BMI, and CYP4F2 Rs 2108622 Genotype

Variable	Total	Mean ± SD
Age (yr)		
20–29	4	21.13 ± 3.07
30–39	12	22.25 ± 7.79
40–49	13	22.08 ± 7.93
50–59	26	20.96 ± 7.08
60–69	15	18.10 ± 6.45
70–79	6	16.17 ± 5.23
80–89	1	7 ± 0.00
BMI (kg/m)		
Underweight (<18.50)	6	17.67 ± 7.23
Normal (18.50–24.90)	48	19.05 ± 5.21
Overweight (>25)	20	23.33 ± 10.15
Obesity (>30)	3	24.00 ± 8.72
***CYP4F2* rs 2108622 genotype**		
CT	47	19 ± 6.42
CC	27	21 ± 7.35
TT	3	33 ± 6.78

Table 4 Results of Bivariate Analysis Between Patients Demographics and Genotype on Warfarin Dose

Patients Demographics		Bivariate Analysis	p-value	Correlation Coefficient	Multivariate Analysis
Genotype	CC	Kruskal–Wallis	0.02*	-	Yes
	CT				
	TT				
Sex	Male	Mann–Whitney	0.16	-	Yes
	Female				
Age (year)	28–80	Spearman's Rank	0.02*	−0.28	Yes
BMI	17–35	Spearman's Rank	0.03*	0.25	Yes

Notes: * $p \leq 0.05$; significant, $p > 0.05$; nonsignificant.

between age, BMI, and warfarin dosage is very weak (correlation coefficient: 0.00–0.30).[23] Specifically, as age increases, the required dose of warfarin decreases. Conversely, as BMI increases, the required dose of warfarin also increases.

Multivariate Analysis

Multivariate analysis aimed to determine the factors associated with warfarin dosing. Multiple linear regression was used to select age, BMI, sex, and *CYP4F2* genotype for the creation of warfarin dosing formula. The results of the multiple linear regression analysis are shown in Table 5.

Table 5 Multiple Linear Regression Analysis Between Age, BMI, Gender, Genotype, and Warfarin Dose

Variable	Beta coefficient	SE (B)	t-value	p-value	Description
Initial Model:					
Sex	0.21	1.49	0.14	0.89	Non-significant
Age	−0.16	0.06	−2.83	0.01*	Significant
BMI	0.54	0.20	2.67	0.01*	Significant
Genotype	3.51	1.30	2.71	0.01*	Significant
Final Model:					
Age	−0.16	0.06	−2.88	0.01*	Significant
BMI	0.54	0.20	2.70	0.01*	Significant
Genotype	3.55	1.27	2.8	0.01*	Significant
Konstanta	12.736	-	0	-	

Notes: * $p \leq 0.05$; significant, $p > 0.05$; nonsignificant, R-squared value: 0.25.

Quality of Life

Quality of life of warfarin therapy patients in Dr. Hasan Sadikin Central General Hospital is presented in Table 4, with categories. The lower score showed a better quality of life and the higher score showed worse conditions. In addition, the results showed that the highest percentage score was included in the category < 56,266. This showed that most patients on warfarin therapy had a better quality of life.

The principle of multiple linear regression analysis used was backward elimination. In the initial model, all variables were entered simultaneously, and those with a significance value >0.05 were excluded. The final model of this regression analysis included three variables, namely age, BMI, and genotype. Table 5 shows that the final model analysis has a significance value of <0.01 for each variable. This result suggests that age ($p = 0.01$), BMI ($p = 0.01$), and genotype ($p = 0.01$) have a significant influence on the determination of warfarin dose.

Based on Table 5, the regression model can be expressed as $y = 12.736–0.160\times1 + 0.540\times2 + 3.545X3$, or dose = 12.736–0.16**age* + *0.54**BMI + 3.55**CYP4F2* genotype, where 1 = CC, 2 = CT, and 3 = TT. The constant 12.736 represents warfarin dose in mg/week when age, BMI, and genotype are not considered. The regression coefficient of −0.16 (β1) shows that for every decrease in age, warfarin dose increases by 0.16 mg/week. The regression coefficient of 0.54 (β2) shows that each unit increase in BMI will raise warfarin dose by 0.54 mg/week. Finally, the regression coefficient of 3.55 (β3) suggests that the presence of the *CYP4F2* C > T polymorphism increases warfarin dose by 3.55 mg/week.

The result in Table 5 showed an R-squared value of 0.25, showing that 25% of the variance in warfarin dose was explained by age, BMI, and *CYP4F2* genotype, while the remaining 75% was determined by other factors not included in this research. The effective contribution of each variable was 8.76%, 8.29%, and 7.95% for age, *CYP4F2* gene polymorphism, and BMI. The effective contribution can be calculated using the formula $SE\% = \beta x \times rxy \times 100\%$.

Discussion

In this research, 77 patients met the inclusion criteria, consisting of 37 men and 40 women, with an average BMI of 23.63 kg/m². The *CYP4F2 rs2108622* gene polymorphism profile included 47 patients with the CC genotype, 27 with CT, and 3 with the TT. Table 3 shows that the older patients, the lower the dose required. The results of this research are consistent with previous reports that patients with middle and old age require warfarin doses 10.60% lower than young age, as the age of patients decreases the weekly dose by 0.40 mg per year of age.[24] In addition, in old age, there are many hemorrhagic events due to the use of drugs that can increase the risk of bleeding, such as antiplatelets, anticoagulants, statins, and amiodarone.[25] The low dose of warfarin in elderly patients was attributed to decreased activity of the vitamin K redox recycling system, which was affected by age-related physiological changes. These changes included alterations in body composition, an increase in fat tissue (leading to an increased volume of distribution for fat-soluble drugs), slowing of metabolic processes, and reduced blood perfusion to the intestinal region.[26,27]

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Dosing based on BMI classification showed that the higher the BMI index, the greater the weekly dose required. The average weekly dose for obese patients was 24 mg, which was 26.38% greater than the underweight and 5 mg higher than normal-weight patients (Table 3). This result was consistent with previous research showing a correlation between weekly dose and BMI. Research by Alshammari et al (2020) and Mueller et al (2014) showed significant results that obese patients require weekly doses 20% higher than those of normal and overweight.[28,29] According to Yoo et al (2012), an increase in body weight was directly proportional to the required warfarin dose and INR value. Patients over 80 years old and weighing less than 55 kg needed a maintenance dose of 3 mg. Meanwhile, those under 55 years old and weighing more than 50 kg required a dose of 10 mg. Patients within these two age and weight ranges needed a dose of 3–7 mg.[30] This is due to differences in pharmacokinetics in obese patients, specifically, in drug distribution within tissues, volume of distribution (Vd), blood flow, plasma protein binding, and drug elimination. The absorption process remains similar to that of normal-weight patients. Obese patients have greater absolute body and fat mass, and the hemodynamic conditions can enhance drug kinetics. Changes in plasma protein-binding concentrations can impact the movement of drugs into tissue compartments, influencing therapeutic effects. Furthermore, the need for larger weekly doses in obese patients was attributed to increased body weight, which affected the volume of distribution and clearance of warfarin, leading to elevated coagulation factors.[31]

Dosing based on the *CYP4F2 rs2108622* genetic polymorphism showed that patients with CC, CT, and TT genotypes required doses of 19 mg, 21 mg, and 33 mg, respectively. The weekly dose for TT patients was significantly greater than CC and CT, as shown in Table 3. Several countries have conducted research on *CYP4F2* polymorphism and the effect on warfarin dosing. Research in China,[32] Iran,[33] Italy,[34] and India[17] showed that patients with the *CYP4F2* polymorphism required higher warfarin doses. However, research conducted on populations in the UK,[35] Japan,[36] and Norway[37] suggested that *CYP4F2* polymorphism had no significant influence on warfarin dosing.

The *CYP4F2* gene expression catalyzes the hydroxylation of vitamin K1 (VK1) into an inactive form, hydroxyvitamin K. This gene served as an important negative regulator of vitamin K levels, thereby affecting blood clotting.[38] The *CYP4F2 rs2108622* V433M variant arises from a polymorphism including the C > T nucleotide substitution. The T allele in *rs2108622* replaced a valine residue with a methionine residue at position 433 in the coding region. This change impacted enzyme activity, and drug metabolism, as well as physiological and pathophysiological processes. The increase in warfarin dose for CT and TT genotypes was consistent with the observed rise in plasma concentration.

Molecular dynamics (MD) research showed that the *CYP4F2* V433M variant was associated with a decrease in protein stability, as evident by free energy values. Free energy values below zero suggested low stability. Destabilization of the protein structure could alter biological function and disrupt signal cascades and normal protein pathways. The V433M variant impacted the physicochemical characteristics, intermolecular interactions, as well as functional and structural properties of the protein. Furthermore, the mutant amino acid (methionine) was larger than the wild-type (valine), leading to structural mismatches within the protein. The wild-type amino acid was located in a critical position for interacting with other molecules that are essential for protein activity. Mutations could disrupt these interactions, affecting the signaling cascade from the binding to the activity domain.[19]

Research by McDonald et al in 2009 showed the participation of *CYP4F2* in the oxidative degradation of vitamin K and oxidative activity. The protein encoded by the *rs2108622* T allele had reduced activity compared to the wild-type in the genotyping of liver microsomal enzymes, with the TT phenotype showing a 75% reduction in vitamin K oxidative activity. The *CYP4F2 rs2108622* V433M variant had a diminished ability to metabolize VK1 to hydroxyvitamin K1, resulting in reduced steady-state hepatic enzyme concentration. Consequently, patients with the *rs2108622* polymorphism tend to have elevated hepatic VK1 levels, leading to a requirement for higher warfarin doses to achieve the same anticoagulant response.[19]

Based on the INR values obtained in this study, the majority of patients with CYP4F2 genotypes CC, CT, and TT had INR values within the target therapeutic range of 2–3. Among the CC genotype group, only 4 patients had INR values exceeding 3, while 3 patients in the CT group exhibited similar results. Notably, no patients with the TT genotype had INR values above 3. These findings suggest that most patients across all genotypes were effectively managed within the desired therapeutic range, reducing the risk of adverse outcomes such as bleeding. Furthermore, there were no reports of

major bleeding events among the study participants, further supporting the safety of the dosing regimens utilized in this population (Table 2).

The algorithm model obtained was y = 12.736–0.160×1 + 0.540×2 + 3.545X3, or dose = 12.736–0.16**age* + *0.54**BMI + 3.55**CYP4F2* genotype, where 1 = CC, 2 = CT, and 3 = TT. The results of this algorithm are consistent with several models developed in various countries, such as in Japan (Dose = 2.263 + 4.248 x (*VKORC1* G/G) + 1.067 x (*VKOCR1* A/G) − 2.416 x (*CYP2C9**3/*3) − 0.864 (x*CYP2C9**1/*3) + 1.308 x *BSA* + 0.025 x age), China (Dose = 0.727–0.007 x age + 0.384 x *BSA* + 0.403 x (*VKORC1* G/A) + 0.554 x (*VKORC1* G/G) − 0.482 x (*CYP2C9**1/*3) − 1.583 x (*CYP2C9**3/*3), Italia (Dose = 7.39764–0.02734 x age + 1.06287 x *BSA* − 1.04468 x *VKORC1* A/G − 2.12117 x *VKORC1*), and USA (Dose = 3.52–0.006 x age + 0.38 x BSA − 0.15 x *hypertension* − 0.23 x (*CYP2C9**1/*3 or *3/*3) − 0.24 x (*VKORC1* A/G) − 0.48 x (*VKORC1*).[22,39,40]

The similarity of the algorithm obtained in this research with those from several other countries was in the inclusion of age and BMI or BSA as factors in the dosing model. The correlation between age and dose was negative across research, namely Japan (+0.025 x age), China (−0.007 x age), Italy (−0.02734 x age), America (−0.006 x age), and Indonesia (−0.16 x age). This result showed that as age increases, the required dose tends to decrease. In contrast, BMI showed a positive correlation, suggesting that the higher the BMI, the greater the required dose. A key difference between the algorithm developed in this research and models from other countries was the genetic factors. While previous investigation focused on *VKORC1* and *CYP2C9*, this research emphasized *CYP4F2*, due to its crucial role in the vitamin K cycle, which was directly related to the vitamin K intake.

The results of this study align with previous findings indicating that age and BMI significantly influence warfarin dosing. For example, Khoury et al (2014) demonstrated that warfarin dosage decreases with age, consistent with our findings.[41] Similarly, the observed correlation between higher BMI and increased warfarin requirements corresponds with results reported by Alshammari et al (2020) and Mueller et al (2014).[28,29] However, our study highlights CYP4F2 as a genetic factor in warfarin dosing, diverging from studies in other countries that emphasize VKORC1 and CYP2C9. This underscores the importance of considering population-specific genetic variations, such as CYP4F2 in Indonesia, in developing dosing algorithms.

The limitations of this research include the relatively small sample size, which may not accurately represent the broader population, thereby limiting the generalizability of the results to all patients with similar conditions. Future research with larger sample sizes is needed to validate these results. Additionally, this research was conducted at only one hospital within a specific geographical area, which could introduce location and population bias, as patients from other regions or hospitals may exhibit different characteristics. Comprehensive analyses that incorporate more genetic factors, as well as other non-clinical variables, are necessary for a more thorough understanding of these issues.

Conclusion

In conclusion, the factors that influenced warfarin dose adjustment in cardiovascular patients in Indonesia were age, BMI, and the *CYP4F2* gene polymorphism *rs2108622*. Specifically, as age increased, the required dose decreased. The *CYP4F2 rs2108622* gene polymorphism also affected warfarin dose variation, with patients carrying the TT polymorphism requiring higher doses. The percentage contributions of each factor to warfarin dose adjustment included 8.76%, 7.95%, and 8.29% for age, BMI, and gene polymorphism, respectively. The total contribution of age, BMI, and *CYP4F2* genotype to warfarin dose adjustment was 25%. Finally, the linear regression model for predicting warfarin dose was represented by the equation y = 12.736–0.16*Age* + *0.54* BMI + 3.55*Genotype. In addition, further exploration of International Normalized Ratio (INR) data could provide more insights into the warfarin response, as INR is a key parameter for monitoring warfarin therapy. The relationship between INR levels and the influencing factors identified in this study may help optimize dosing strategies for cardiovascular patients in Indonesia.

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Disclosure

The authors report no conflicts of interest in this work.

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