

Validating two international warfarin pharmacogenetic dosing algorithms for estimating the maintenance dose for patients in Singapore

Dear Editor,

Predicting optimal warfarin dosing is difficult due to complex pharmacodynamics and pharmacokinetics, narrow therapeutic index and susceptibility to many factors.¹ Genetic variations of the CYP2C9 and VKORC1 enzymes, occurring in different frequencies in different populations, play a significant role in determining warfarin dosing.¹⁻⁴ Using pharmacogenetic dosing algorithms to predict warfarin doses may shorten the time to achieve target International Normalised Ratio (INR) and stable dose.^{2,5} The Clinical Pharmacogenetics Implementation Consortium Guidelines 2017 Update⁴ recommends the Gage (WarfarinDosing.org⁷) and International Warfarin Pharmacogenetics Consortium (IWPC)⁸ pharmacogenetic algorithms.

Singapore's genetic make-up is diverse due to its multi-ethnic community that consists of Chinese, Malays, Indians and other ethnicities. We present our evaluation of the Gage and IWPC algorithms in estimating warfarin maintenance doses in Singapore.

Patients receiving warfarin at a large hospital in Singapore's outpatient anticoagulation clinics were prospectively recruited between March 2020 and June 2021. Patients were ≥ 21 years, have an INR target between 2 and 3, and are stable on therapeutic warfarin doses beyond a month, derived with the hospital's standardised clinical dosing algorithm. We excluded patients with unstable liver or renal disease, end-stage renal failure not on regular dialysis, organ transplant within 2 months, or have received short-term drugs that interact with warfarin.

Consented patients' blood or buccal swab samples were analysed for CYP2C9 (rs1799853), VKORC1 (rs9923231) and CYP4F2 (rs2108622) polymorphisms using Taqman SNP Genotyping Assays on real-time Polymerase Chain Reaction systems.

Patients' demographics, warfarin profile, concomitant interacting drugs, smoking/alcohol history, vitamin K intake, health/herbal supplement use and physical activity were collected. Clinical and genotype data (CYP2C9 [*1, *2 and *3]), VKORC1 c.-1639G>A allele and CYP4F2*3 were entered into the Gage and IWPC pharmacogenetic dosing algorithms to obtain the predicted warfarin doses. Warfarin doses were expressed as total

dosage per week. Descriptive analyses were performed using SPSS Statistics 28.

The proportion of patients whose predicted weekly warfarin maintenance dose differ $\pm 20\%$ of the actual weekly maintenance dose was determined. Bland and Altman analysis was used to study the agreement between the observed and the predicted doses, limits of agreement set as 95% of the data.

Ninety patients were recruited. The actual, Gage-predicted and IWPC-predicted mean warfarin maintenance doses of these patients were 27.2 ± 14.9 mg/week, 28.5 ± 10.9 mg/week and 27.4 ± 10.5 mg/week, respectively. The mean differences between actual and algorithm-predicted doses were -1.3 ± 10.5 mg/week (95% confidence interval [CI] -3.45 to 0.93) with Gage and -0.1 ± 9.7 mg/week (95% CI -2.16 to 1.92) with IWPC, respectively. Though not statistically different, the IWPC performed marginally better, with 45.6% of patients falling within $\pm 20\%$ of their actual doses than the 41.1% with the Gage algorithm, despite not requiring CYP4F2 genetic variation data, a contrast with Chan et al. who proposed that polymorphisms in this gene have a bearing on warfarin dose variation.⁶ It is noteworthy that IWPC algorithm included Asian population data.^{8,9}

Seven outliers were identified. Five required very high warfarin doses (includes 1 with 28 vegetable servings/week [versus average 11]), and 3 Indians on carbamazepine or azathioprine. Remaining 2 have VKORC1 G/G and at least 1 CYP4F2 *3 variant but required significantly lower warfarin doses. Table 1 shows genotypic variants in different ethnic groups.

Of the 31 Chinese patients, 11 (35.5%) and 15 (48.4%) of them were within $\pm 20\%$ difference of their Gage and IWPC predictions, respectively. The mean dose differences between actual and algorithm-predicted doses were -1.9 ± 7.4 mg/week (95% CI -4.58 to 0.82) for Gage, and -0.1 ± 7.6 mg/week (95% CI -2.82 to 2.72) for IWPC.

Fourteen (50.0%) of the 28 Indian patients' actual warfarin doses were within $\pm 20\%$ difference of their Gage or IWPC predictions. Mean dose differences between actual and algorithm-predicted doses were 1.0 ± 14.8 mg/week (95% CI -4.76 to 6.72) for Gage, and 2.6 ± 12.2 mg/week (95% CI -2.08 to 7.37) for IWPC, respectively.

Table 1. Frequency of the genotypic variants and their respective weekly warfarin maintenance dose requirements in different ethnic groups.

		Chinese (n=31) no. (%)	Malay (n=31) no. (%)	Indian (n=28) no. (%)	Total (n=90) no. (%)
Warfarin maintenance dose, mean $\pm$ SD (mg/week)		20.8 $\pm$ 9.5	24.8 $\pm$ 12.6	37.1 $\pm$ 17.4	27.2 $\pm$ 14.9
VKORC1					
-1639 A/A	18.9 $\pm$ 6.5	24 (77.4)	16 (51.6)	2 (7.1)	42 (46.7)
-1639 G/A	29.9 $\pm$ 12.5	7 (22.6)	13 (41.9)	8 (28.6)	28 (31.1)
-1639 G/G	41.1 $\pm$ 19.2	0	2 (6.5)	18 (64.3)	20 (22.2)
CYP2C9					
*1/*1	27.4 $\pm$ 15.6	31 (100)	28 (90.3)	17 (60.7)	76 (84.4)
*1/*2	-	0	0	1 (3.6)	1 (1.1)
*1/*3	25.3 $\pm$ 11.1	0	3 (9.7)	9 (32.1)	12 (13.3)
*2/*2	-	0	0	0	0
*2/*3	-	0	0	1 (3.6)	1 (1.1)
*3/*3	-	0	0	0	0
CYP4F2					
*1/*1 (V433M CC)	24.9 $\pm$ 12.6	14 (45.2)	17 (54.8)	8 (28.6)	39 (43.3)
*1/*3 (V433M CT)	29.4 $\pm$ 17.5	14 (45.2)	14 (45.2)	15 (53.6)	43 (47.7)
*3/*3 (V433M TT)	26.9 $\pm$ 8.55	3 (9.7)	0	5 (17.9)	8 (8.9)

Chinese patients require the lowest maintenance dose, with 100% of the group possessing the CYP2C9*1/*1 allele, and 77.4% the VKORC1 -1639 A/A allele. Indian patients show heterogeneity in the CYP2C9 and VKORC1 alleles, predicating the largest spread of warfarin doses and have the highest maintenance doses (64.3% have the VKORC1 -1639 G/G allele).

SD: standard deviation

Twelve (38.7%) of the 31 Malay patients' actual warfarin doses were within $\pm 20\%$ difference of their Gage or IWPC predictions. Mean dose differences between actual and predicted were -2.7 ± 8.1 mg/week (95% CI -5.64 to 0.32) for Gage, -2.7 ± 8.7 mg/week (95% CI -5.88 to 0.53) for IWPC, respectively.

Dose groups were categorised into low-dose (<21 mg/week), moderate-dose (21 – 34.5 mg/week) and high-dose (≥ 35 mg/week), based on our clinical experience.

Among the 37 patients in the low-dose group, 7 (18.9%) and 12 (32.4%) actual doses were within $\pm 20\%$ difference of their Gage and IWPC predictions, respectively. Mean actual doses were significantly different from Gage-predicted -6.3 ± 4.5 mg/week (95% CI -7.84 to -4.86) and IWPC-predicted -5.5 ± 5.1 mg/week (95% CI -7.15 to -3.77) doses. The algorithms overestimated the doses by ≥ 5 – 6 mg/week.

Among the 23 patients in the high-dose group, 10 (43.5%) and 9 (39.1%) actual doses were within $\pm 20\%$ difference of their Gage- and IWPC-predicted, respectively. Mean actual doses were significantly different when compared with both Gage-predicted 8.7 ± 12.0 mg/week (95% CI 3.52 – 13.88) and IWPC-predicted 9.7 ± 10.2 mg/week (95% CI 5.28 – 14.07) doses. The dosing algorithms tend to underestimate doses by a mean of 8 – 9 mg/week, with 8 patients having high warfarin doses of ≥ 7 mg/day. A systematic review also showed that 22 dosing algorithms (including Gage) tend to underestimate when doses exceed 7 mg/day.¹⁰

For the 30 patients in the moderate dose group, 20 (66.7%) of them were within $\pm 20\%$ difference compared with their predicted doses. The mean differences were -2.6 ± 9.4 mg/week (95% CI -6.13 to 0.90) when compared with Gage-predicted, and -1.0 ± 8.2 mg/week (95% CI -4.10 to 2.03) with IWPC-predicted doses.

The pharmacogenetic dosing algorithms were originally developed to predict warfarin initiation doses. However, as we cannot take into account the full variability determining the initiation doses, we compared the predicted doses to maintenance doses and allowed for 20% difference.⁸ Patients with unstable renal and liver conditions (and inherently higher bleeding risk) were excluded from the study, hence a more conservative approach is needed if these dosing algorithms are used.

Overall, there is no significant difference between the Gage- or IWPC-predicted doses compared with the observed maintenance doses.

Integrating pharmacogenetic algorithms into local clinical practice is promising, as it can potentially enable faster and safer attainment of therapeutic anticoagulation. Further studies can be done to identify patient groups who will benefit most from genetic testing.

Acknowledgements

We wish to express our sincere thanks to the Tan Tock Seng Hospital Anticoagulation Pharmacist team, and our research coordinators Ms Felicia Lee Si Min and Ms Stephanie Wong Li Ling from Clinical Research & Innovation Office (CRIO), for their support and guidance in the study process and patient recruitment.

Funding and ethics

This study was approved by the NHG Domain Specific Review Board (2019/01166) and supported by the Personalised Medicine Translational Research Seed Funding Programme (PMTRSP19-03).

Keywords: algorithm, pharmacogenetics, pharmacogenomics, Singapore, warfarin

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