Helin Tuukka Antero (Orcid ID: 0000-0002-5273-8088)
Webb Andrew (Orcid ID: 0000-0002-8109-1877)

Warfarin dose requirement in patients having severe thrombosis or thrombophilia

PI: Prof Riitta Lassila, MD, PhD

Running head: Warfarin dosing algorithm in thrombophilia

Authors: Tuukka A. Helin[1], Lotta Joutsi-Korhonen[1], Heidi Asmundela[2] Mikko Niemi[3], Arto Orpana[4], and Riitta Lassila[1,2]

1. Coagulation Disorders Unit, Clinical Chemistry, University of Helsinki and HUSLAB, Helsinki University Hospital, POB 720, 00029, Helsinki, Finland
2. Coagulation Disorders Unit, Hematology, University of Helsinki, Helsinki University Hospital, POB 320, 00029 Helsinki. Finland
3. Department of Clinical Pharmacology, University of Helsinki and HUSLAB, Helsinki University Hospital, POB 320, 00029 Helsinki. Finland
4. Genetics and Clinical Chemistry, University of Helsinki and HUSLAB, Helsinki University Hospital, POB 320, 00029 Helsinki. Finland

Submitting author: Tuukka A. Helin email: tuukka.helin@hus.fi
Corresponding author: Riitta Lassila, email: riitta.lassila@hus.fi
PI: Prof Riitta Lassila, MD, PhD

The authors confirm that the PI for this paper is Riitta Lassila and that she had direct clinical responsibility for the patients

Keywords: warfarin, thrombosis, thrombophilia, *CYP2C9, VKORC1*, dosing algorithm
Number of tables: 4
Number of figures: 2
Word count: 2873

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bcp.13948

ABSTRACT

AIMS

Warfarin dose requirement varies significantly. We compared the clinically established international normalized ratio (INR) -based doses among patients with severe thrombosis and/or thrombophilia with estimates from genetic dosing algorithms.

METHODS

Fifty patients with severe thrombosis and/or thrombophilia requiring permanent anticoagulation, referred to the Helsinki University Hospital Coagulation Center, were screened for thrombophilias and genotyped for *CYP2C9*2* (c.430C>T, rs1799853), *CYP2C9*3* (c.1075A>C, rs1057910) and *VKORC1* c.-1639G>A (rs9923231) variants. The warfarin maintenance doses (target INR 2.0-3.0 in 94%, 2.5-3.5 in 6%) were estimated by the Gage and the International Warfarin Pharmacogenetics Consortium (IWPC) algorithms. The individual warfarin maintenance dose was tailored, supplementing estimates with comprehensive clinical evaluation and INR data.

RESULTS

Mean patient age was 47 years (range, 20-76), and BMI 27 (SD 6), 68% being women. Forty-six (92%) had previous venous or arterial thrombosis, and 26 (52%) had a thrombophilia, with 22% having concurrent aspirin. A total of 40% carried the *CYP2C9*2* or **3* allele and 54% carried the *VKORC1* -1639A allele. The daily mean maintenance dose of warfarin estimated by the Gage algorithm was 5.4 mg (95% CI 4.9-5.9 mg,), and by the IWPC algorithm was 5.2 mg (95% CI 4.7-5.7 mg,). The daily warfarin maintenance dose after clinical visits and follow-up was higher than the estimates, mean 6.9 mg (95% CI 5.6-8.2 mg, $p<0.006$), with highest dose in patients having multiple thrombophilic factors ($p<0.03$).

CONCLUSIONS

In severe thrombosis and/or thrombophilia, variation in thrombin generation and pharmacodynamics influences warfarin response. Pharmacogenetic dosing algorithms seem to underestimate dose requirement.

What is already known about this subject

- Effective warfarin dose has high inter-individual variability, owing in part to genetic variations in *CYP2C9* and *VKORC1*
- Warfarin dosing algorithms accounting for genetic and clinical factors may improve warfarin therapeutic efficacy, diminishing thromboembolic and bleeding complications
- Among thrombophilia patients, risks for complications are higher

What this study adds

- Thrombophilia patients are significantly younger than the warfarin using patients on average
- Dosing algorithms seem to underestimate the daily warfarin maintenance dose among patients having thrombophilia and/or severe thrombosis

Introduction

Vitamin K antagonists (VKAs) impair the synthesis of vitamin K-dependent coagulation factors (II, VII, IX and X) by inhibiting the conversion of vitamin K epoxide to its active form via the vitamin K epoxide reductase complex (VKOR). Therefore, warfarin and other VKAs are used as anticoagulants for treatment or prevention of thromboembolism and in association with atrial fibrillation (AF), prosthetic heart valves and thrombophilia. To reach the efficient and safe level of anticoagulation, warfarin dosing is individually tailored, and regular follow-up is required by laboratory measurements of International Normalized Ratio (INR) based on the prothrombin time with the Owren reagent [1]. The average daily warfarin dose for all indications is approximately 5 mg, while individual doses may vary even more than 10-fold [1,2]

Many factors, including, patient diet, smoking, alcohol use, other medications and genetic variants influence warfarin dose requirement [3,4]. It is common practise to start warfarin with a fixed dose and tailor the individual dose with INR monitoring. With this approach, the time to achieve INR treatment target level might be prolonged in patients at the either extremes of the dose requirements. Clinical algorithms aim at predicting the required warfarin dose, facilitating the achievement of the INR target level. The common factors affecting warfarin dose in the algorithms include: age, race, body surface area, smoking, amiodarone and statin use [5].

Genetic variants influence the efficacy of warfarin by either a pharmacokinetic or pharmacodynamic mechanism [6]. Warfarin is a racemic mixture of the S- and R-enantiomers. The pharmacologically more active S-warfarin is metabolized mainly by

CYP2C9, and common *CYP2C9* variants, such as *CYP2C9*2* (c.430C>T, rs1799853), and *CYP2C9*3* (c.1075A>C, rs1057910), impair S-warfarin metabolism, reducing warfarin dose requirement [7]. In addition, variations of the *VKORC1*-gene impact the activity of the VKOR enzyme, and the *VKORC1*-variant c.-1639G>A (rs9923231) increases warfarin sensitivity [8,9]. Both the *CYP2C9* and *VKORC1* variants are common among individuals of Caucasian ancestry and contribute to the individual warfarin dose requirement [10-14]. The common *VKORC1* and *CYP2C9* variants account for up to 27% and 18%, respectively, of the variability in stable warfarin dose requirement among Caucasians [6,11].

Patient's genetic profiling can be used to predict warfarin dosage and multiple algorithms incorporating both genotypic, and clinical data exist to predict warfarin doses [6],according to the Gage algorithm and the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm. They are commonly adopted for this purpose in clinical practice, and are available at warfarindosing.org. [1,15]. Pharmacogenetic dosing algorithm for warfarin has been shown to reduce the time to reach the stable dose, improve the percent time in therapeutic range (TTR) and decrease the number of episodes with an INR above 4 in a homogenous European population [16]. The potential benefit of using genetic profiling is likely substantial in cases of increased risks of 1) bleeding complications or 2) thrombosis, when direct oral anticoagulants are not recommended due to the uncertainty in dose responses and drug exposure, and 3) when therapeutic warfarin dosages must be reached quickly. Our aim was to characterize patients having a history of severe thrombosis and/or thrombophilia, and to examine the accuracy of genetic and algorithmic estimates of warfarin doses among these challenging patients.

Methods

This was a retrospective, register-based study on patients referred to the Coagulation Disorders Unit in the Helsinki University Hospital due to severe thrombosis and/or thrombophilia to be treated with long-term or permanent warfarin anticoagulation. Warfarin genotyping was routinely performed for patients in whom permanent anticoagulation was indicated, and thus retrospective analysis of the data was possible. Genetic testing for single nucleotide polymorphisms (SNPs) affecting warfarin metabolism has been available for clinical use since October 2007. Ethics board was consulted, and ethics permit was not requested due to the register-based nature of the study, as mandated by the Finnish law [17]. Patient data were pseudonymized and patients were not contacted. The Helsinki University Hospital research permit was granted for the research protocol (decision number 20/2018). Patients referred during 2009-2018 were assessed for inclusion in the study. At least one of the following criteria had to be met for inclusion: thrombosis at a young age (under 50 years), strong family history of thrombosis, known severe thrombophilia in laboratory screen (phospholipid antibodies, antithrombin, protein C or S deficiency, *FV Leiden* or *FII G20210A* variant homozygosity or combined heterozygosity for the last two), unusual site of thrombosis, multiple spontaneous thromboses or miscarriages, both arterial and venous thromboses, active cancer and thrombosis, valvular replacement or other cardiac insufficiency predisposing to thrombosis. These criteria indicate permanent anticoagulation and were based on the recommendations on testing for thrombophilia [18,19]. Altogether 50 patients met the criteria, with 19 patients (38%) meeting a single inclusion criterium, but 31 patients (62%) carried two or more criteria. Laboratory thrombophilia screen had been performed for 47 patients, including thrombin time, lupus anticoagulant, beta-2-glycoprotein and cardiolipin antibodies, *FV Leiden*, *FII G20210A* variant, antithrombin activity, protein C activity, protein S free antigen and FVIII:C assays. Thrombophilia screen had been assessed

in 41 patients before, in 4 patients simultaneously with, and in 2 patients after pharmacogenetic testing for warfarin. Genotyping for *CYP2C9*2, CYP2C9*3* and *VKORC1* c.-1639G>A variants and genotyping for *FV Leiden* and *FII G20210A* variant in the thrombophilia panel had been performed with an automated cyclic mini-sequencing method (HUSLAB, Helsinki University Hospital, Finland).

The INR target level in most patients was set to 2.0-3.0 INR, while 3 patients (6%) with valvular replacement had an INR target level of 2.5-3.5. Initially, patients were genotyped for warfarin associated SNPs and warfarin dose was estimated with the local algorithm based on the study by Wadelius et al [20]. After genotyping, all patients had been called for a clinical visit and subsequent follow-up visits. If patient met the INR target the current warfarin dose in use already after several weeks of stable dose was recorded (real-life dose). If the INR target was not met, the subsequent warfarin dose was recommended based on a combination of genotypic, clinical, calculated algorithmic and INR data, where the minimum warfarin dose was obtained from the local algorithm. The patient was followed up until a new stable dose was achieved. Each patient was followed for several months at the clinic to verify the stabilization of the dose needed. This final, prescribed stable warfarin dose was recorded and is henceforth referred to as the real-life dose of the patient. Subsequently, the Gage and IWPC algorithms were used to estimate warfarin dose based on genetic and clinical data and compared with the real-life dose [1,15].

The different dose estimates were compared with one another using Wilcoxon signed rank test, and different patient groups were compared to one another with Kruskal-Wallis H test, with IBM SPSS® statistics program (version 22). The median difference and 95% confidence intervals were estimated with Hodges-Lehmann test [21]. The differences were considered

statistically significant when *p* was below 0.05.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to Pharmacology.

Results

Patient characteristics

The average age of the patients was 47 years (range 20-76), and the mean BMI was 27 kg/m^2 (SD 6). Of all patients, 24/50 (48%) had thrombosis at age under 50 years. Despite the history of thrombosis and/or thrombophilia, four patients (8%) continued smoking. ASA use was prevalent, with 11 patients (22%) having ASA concurrent with warfarin due to history of arterial thrombosis (Table 1). Most patients, 46 (92%), had thrombosis as the primary indication for warfarin treatment, with the remaining four patients (8%) having valvular replacement as the primary indication, three of them having the higher INR target level of 2.5-3.5 due to the replacement in the mitral valve position (Table 2). All the valvular replacement patients also had additional factors predisposing to thrombosis– namely arteriosclerosis, pulmonary hypertension, heart malformation or insulin-dependent diabetes. In over half of the patients screened in the laboratory, 26 (55%) carried thrombophilia, most commonly *FV Leiden* (24%), while seven patients (14%) had more than one thrombophilia (Table 3). Of the patients who had thrombosis 25/46 (54%) presented with a thrombophilia. Three patients with valvular replacement as their warfarin indication were not tested for thrombophilia, while one of them had an elevated FVIII (>190 IU/dL). Fourteen (28%) patients had the reference *CYP2C9*1/*1* and *VKOCR1* c.-1639GG genotypes, 20 (40%) carried either *CYP2C9*2, *3* or both and 27 (54%) carried the *VKORC1 c.-1639G>A* variant

(Table 4). None of the patients used the enzyme inhibitor or inducer medicines included in the IWPC algorithm. The enzyme inhibitor or inducer medicines included in the algorithm are azoles (e.g. fluconazole), amiodarone, sulfonamid, phenytoin, carbamazepine and rifampicin.

Warfarin doses

Daily warfarin doses estimated by the Gage algorithm (mean 5.4 mg, 95% CI 4.9-6.0 mg, SD 2.0 mg, range 1.5-9.3 mg) or by the IWPC algorithm (mean 5.2 mg, 95% CI 4.7-5.7 mg, SD 1.9 mg, range 0.9-8.3 mg) were significantly lower than the observed real-life warfarin dose (mean 6.9 mg, 95% CI 5.6-8.2 mg, SD 4.5 mg, range 0.8-30.0 mg). Real-life daily dose median difference to Gage algorithm was 1.2 mg (95% CI 0.4-2.0 mg, p=0.005) and median difference to IWPC algorithm was 1.4 mg (95% CI 0.5-2.1 mg, p=0.001). The correlation between the dose estimates according to various algorithms and the established doses in real-life was poor (Figure 1). In some patients the real-life warfarin dose requirement was higher than predicted, up to 20 mg higher daily dose than that predicted by the algorithms (Figure 2). The patient with the highest daily warfarin dose of 30 mg was a 35 yo woman who had DVT and FV Leiden mutation. The patient was not overweight (BMI 24), did not smoke and had a normal genotype for *CYP2C9* and *VKORC1*. No enzyme inducer medication was present. The cause of the very high required warfarin dose remains unknown to us. In particular, the real-life warfarin daily dose was higher in patients with two or more admission criteria as opposed to patients with a single admission criterion (mean 7.9 mg vs. 5.3 mg, median difference 2.0 mg, 95 % CI 0.1-4.0, p<0.03). Warfarin dose requirements were the highest in patients after an ischemic stroke without AF, valvular replacement, or deep vein thrombosis (Table 2), however, these differences did not reach statistical significance (p=0.22). Statin use, ASA use, smoking or BMI did not appear to influence the warfarin dose (data not shown).

Discussion

The pharmacogenetic IWPC and Gage dosing algorithms significantly under-predicted the actual stable warfarin doses in these highly thrombogenic patients. In some trials, the use of genotyping among AF patients in the dose estimation has reached the therapeutic warfarin levels faster, and the bleeding risk is diminished compared with the conventional dosing [1,22,23]. These include the randomized controlled trial (RCT) EU-PACT, in which a homogenous European population was studied [24]. To the best of our knowledge, this is the first study to examine warfarin dose in young patients with severe thrombosis and/or thrombophilia. The previously published studies with young patients (<50 years) have been patients with valvular replacement or FV Leiden heterozygous mutation [25-28]. In our study, we included only those forms of thrombophilia, which indicate permanent anticoagulation therapy, and FV Leiden heterozygosity solely was not an inclusion criterion – instead homozygosity or double heterozygosity with FII mutation was required. Currently, however, the algorithms accounting for genotypic and clinical factors in comparison with the algorithms accounting for the clinical factors only, have not shown consistent results favouring genotypic algorithms. In the RCT COAG, in a heterogenous population (27% were people of African ancestry) the genetic data added to the clinical algorithm did not improve treatment outcome, while in the Gage trial, genotype -guided dosing improved outcome with regard to thrombosis [5,22,29]. In the highly thrombogenic patients, the accurate initial dose estimation might improve outcome, as risks for thrombotic complications are higher, albeit heparin is used until the INR target is well reached. Here, the average daily warfarin dose in our thrombotic or thrombophilic patients, was on average 1.9 mg higher than in the literature including patients with all warfarin indications (6.9 mg vs. 5 mg) [1]. Our results are consistent with a recent meta-analysis, where it was reported that with daily warfarin doses of over 7 mg, the dose prediction algorithms underestimated the maintenance dose [30]. In our

study, the patients with more than one admission criteria, had significantly higher warfarin dose requirement than those with only one admission criterion, highlighting the increased thrombogenic potential. It also seemed that the valve replacement patients had a higher dose demand, corresponding to the increased INR target range of 2.5-3.5, but patients with the history of non-AF ischemic stroke or deep vein thrombosis and pulmonary embolism as well seemed to need a higher than average warfarin dose, although statistical significance was not reached due to small sample size. Yet, the current dose prediction algorithms, validated in AF patients, seem to underestimate the required warfarin dose in thrombogenic patients as a group, and especially in patients with multiple thrombogenic factors. It has been shown, that in patients with antiphospholipid antibody syndrome, the warfarin dose requirement is increased, relative to inherited thrombophilias and irrespective of the *VKORC1* and *CYP2C9* genotype, suggesting that among thrombophilia patients factors other than those accounted for in the dose algorithms impact the warfarin dose [31]. It has been shown, that in cardiac surgery patients, novel pharmacokinetic-pharmacodynamic models may improve dosing accuracy, particularly at the early stage of dosing (before steady state), taking into account the previous doses and INR responses [32]. However, in comparison with our patient population, cardiac surgery may influence the mechanisms of coagulation and hemodynamics in distinct manners.

Thrombophilias significantly increase the risk of venous thromboembolism, with VTE risk increased by up to 6-fold with antiphospholipid antibodies, 5-fold with increased FVIII:C levels, 5-50 times increased with AT deficiency, which confers the highest risk, 5 times with protein S and C deficiencies, 3-5 times with a heterozygous *FV Leiden* variant, and 2 times with a heterozygous *FII G20210A* variant [33-37]. In this study, only four (8%) of the patients tested for thrombophilia had AT, PC or PS deficiency, which carry the highest

thrombotic risk. The typical warfarin treatment target of INR is 2.0-3.0 applies also in these detected thrombophilia patients according to the laboratory panel.

Good quality warfarin management is essential due to the otherwise increased complication risks. As the typical TTR in all warfarin patients are only approximately 64%, vigilance is required with these patients, keeping in mind that the best results are usually achieved in warfarin self-management patients (up to 72% TTR) [38]. Thrombophilia patients express enhanced responses in thrombin generation assays, likely explaining the higher warfarin dose requirement in these patients [39-43].

Patients were young, on average 47 years of age, whereas in all indications, most warfarin patients are over 60 years of age [44]. Increasing age has been associated with decreasing warfarin doses, which is appreciated in the dosing algorithms. Although the pharmacokinetics of warfarin is similar in young and elderly adults, older patients have lower average weight, increasing their warfarin sensitivity, and also the prothrombin time ratio is increased, while adjusted to warfarin dose, suggesting increased pharmacodynamic effects as well [44,45]. BMI influences the mean daily warfarin dose, although large interindividual variations exist within a BMI group [46]. In our limited sample, with mean BMI only moderately elevated, no effect of BMI was observed on the warfarin dose requirement. The effect of age and BMI (through body surface area) are included in the Gage and IWPC algorithms [1,15]. However, in our study cohort, significantly younger than most warfarin users, the model did not achieve the actual real life dose requirement.

The limitations of this study include the restricted sample size of 50 patients due to a single-site nature of this study. The study was exploratory without power calculations, but

significant differences between algorithms were identified [47]. The findings need to be confirmed in a larger patient populations to enable firmer conclusions. While warfarin indications were recorded (Table 2), in the small subgroups we were unable to analyse the effect of the clinical indication to warfarin dosing. Another major limitation is, that neither the clinician, nor the patient was blinded to the warfarin dose, or to the results of the pharmacogenetic analysis. This might have impacted the warfarin prescription, but on the other hand, this directly reflects the real-life practice. The strengths include the highly thrombogenic non-AF patient cohort in the need of permanent anticoagulation, in which warfarin genotyping has not earlier been systematically evaluated. Warfarin dose was estimated through the warfaringdosing.org website using Gage and IWPC algorithms, the tools that are available to most clinicians, increasing the implications of this study.

Conclusions

Currently, the most used pharmacogenetics dosing algorithms underestimate the warfarin dose required for effective anticoagulation in thrombogenic young (age less than 50 years) patients, particularly those who are at the highest thrombotic risk. A thorough clinical assessment is required in these patients for the effective and safe warfarin anticoagulation which is deemed lifelong. Whether thrombogenic patients will benefit from the other anticoagulation strategies than warfarin remains to be seen in future. Today warfarin, the traditional anticoagulation modality, is recommended for these patients based on its longstanding experience [48].

Acknowledgements

We thank RN Maria Patronen and RN Salla Valtonen in the Coagulation Disorders Unit for their kind contribution in collecting and organizing the patient data from the patient files.

Conflicts of interest statement:

TH, LJK, HA, MN, AO and RL disclose no conflicts of interest.

References

1. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009;360:753-764.
2. James AH, Britt RP, Raskino CL, Thompson SG. Factors affecting the maintenance dose of warfarin. J Clin Pathol 1992;45:704-706.
3. Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, et al. Systematic overview of warfarin and its drug and food interactions. Arch Intern Med 2005;165:1095-1106.
4. Nathisuwan S, Dilokthornsakul P, Chaiyakunapruk N, Morarai T, Yodting T, Piriyachananusorn N. Assessing evidence of interaction between smoking and warfarin: a systematic review and meta-analysis. Chest 2011;139:1130-1139.
5. Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, et al. A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 2013;369:2283-2293.
6. Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. Clin Pharmacol Ther 2017;102:397-404.
7. CYP2 family: CYP2C9. Last modified on 16/09/2015. Accessed on 26/02/2019. IUPHAR/BPS Guide to pharmacology. 2015; Available at: http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1326. Accessed 02/26, 2019.
8. Oxidoreductases: vitamin K epoxide reductase complex subunit 1. Last modified on 05/02/2016. Accessed on 26/02/2019. IUPHAR/BPS Guide to pharmacology. 2016; Available at: http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2645. Accessed 02/26, 2019.

9. Alexander SPH, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD, et al. The Concise Guide to pharmacology 2017/18.. Br J Pharmacol 2017;174 (Suppl 1):S1-S446.
10. Daly AK, Day CP, Aithal GP. CYP2C9 polymorphism and warfarin dose requirements. Br J Clin Pharmacol 2002;53:408-409.
11. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009;113:784-792.
12. Owen RP, Gong L, Sagreiya H, Klein TE, Altman RB. VKORC1 pharmacogenomics summary. Pharmacogenet Genomics 2010;20:642-644.
13. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005;352:2285-2293.
14. Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C, et al. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. Pharmacogenomics 2004;5:895-931.
15. Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008;84:326-331.
16. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013;369:2294-2303.
17. Finnish Information Centre for Register Research
Applying for permission to use register data from THL or other agencies under the Ministry of Social Affairs and Health. Available at: https://rekisteritutkimusen.wordpress.com/data-protection-and-permissions/application-to-thl-or-msh/. Accessed 03/02, 2019.
18. Connors JM. Thrombophilia Testing and Venous Thrombosis. N Engl J Med 2017;377:1177-1187.
19. Eichinger S. Cancer associated thrombosis: risk factors and outcomes. Thromb Res 2016;140 Suppl 1:S12-7.
20. Wadelius M, Chen LY, Eriksson N, Bumpstead S, Ghori J, Wadelius C, et al. Association of warfarin dose with genes involved in its action and metabolism. Hum Genet 2007;121:23-34.
21. Hodges J,L.Jr, Lehmann E,L. Estimates of Location Based on Rank Tests. Ann Math Statist 1963;34
22. Gage BF, Bass AR, Lin H, Woller SC, Stevens SM, Al-Hammadi N, et al. Effect of

Genotype-Guided Warfarin Dosing on Clinical Events and Anticoagulation Control Among Patients Undergoing Hip or Knee Arthroplasty: The GIFT Randomized Clinical Trial. JAMA 2017;318:1115-1124.
23. Tse G, Gong M, Li G, Wong SH, Wu WKK, Wong WT, et al. Genotype-guided warfarin dosing vs. conventional dosing strategies: a systematic review and meta-analysis of randomized controlled trials. Br J Clin Pharmacol 2018;84:1868-1882.
24. Pirmohamed M, Wadelius M, Kamali F, EU-PACT Group. Genotype-guided dosing of vitamin K antagonists. N Engl J Med 2014;370:1764-1765.
25. Huang SW, Chen HS, Wang XQ, Huang L, Xu DL, Hu XJ, et al. Validation of VKORC1 and CYP2C9 genotypes on interindividual warfarin maintenance dose: a prospective study in Chinese patients. Pharmacogenet Genomics 2009;19:226-234.
26. Wang M, Lang X, Cui S, Fei K, Zou L, Cao J, et al. Clinical application of pharmacogenetic-based warfarin-dosing algorithm in patients of Han nationality after rheumatic valve replacement: a randomized and controlled trial. Int J Med Sci 2012;9:472-479.
27. Harikrishnan S, Koshy L, Subramanian R, Sanjay G, Vineeth CP, Nair AJ, et al. Value of VKORC1 (-1639G>A) rs9923231 genotyping in predicting warfarin dose: A replication study in South Indian population. Indian Heart J 2018;70 Suppl 3:S110-S115.
28. Nahar R, Saxena R, Deb R, Verma IC. Pharmacogenetic typing for oral anti-coagulant response among factor V Leiden mutation carriers. Indian J Hum Genet 2012;18:326-331.
29. Kimmel SE. Warfarin pharmacogenomics: current best evidence. J Thromb Haemost 2015;13 Suppl 1:S266-71.
30. Saffian SM, Duffull SB, Wright D. Warfarin Dosing Algorithms Underpredict Dose Requirements in Patients Requiring >/=7 mg Daily: A Systematic Review and Meta-analysis. Clin Pharmacol Ther 2017;102:297-304.
31. Ames PRJ, Margaglione M, Ciampa A, Colaizzo D, Ferrara F, Iannaccone L, et al. Increased warfarin consumption and residual fibrin turnover in thrombotic patients with primary antiphospholipid syndrome. Thrombosis Research 2011;127(6):595-599.
32. Xue L, Holford N, Ding XL, Shen ZY, Huang CR, Zhang H, et al. Theory-based pharmacokinetics and pharmacodynamics of S- and R-warfarin and effects on international normalized ratio: influence of body size, composition and genotype in cardiac surgery patients. Br J Clin Pharmacol 2017;83:823-835.
33. Jenkins PV, Rawley O, Smith OP, O'Donnell JS. Elevated factor VIII levels and risk of

venous thrombosis. Br J Haematol 2012;157:653-663.
34. Zeng W, Tang L, Jian XR, Li YQ, Guo T, Wang QY, et al. Genetic analysis should be included in clinical practice when screening for antithrombin deficiency. Thromb Haemost 2015;113:262-271.
35. Bucciarelli P, Rosendaal FR, Tripodi A, Mannucci PM, De Stefano V, Palareti G, et al. Risk of venous thromboembolism and clinical manifestations in carriers of antithrombin, protein C, protein S deficiency, or activated protein C resistance: a multicenter collaborative family study. Arterioscler Thromb Vasc Biol 1999;19:1026-1033.
36. Folsom AR, Cushman M, Tsai MY, Heckbert SR, Aleksic N. Prospective study of the G20210A polymorphism in the prothrombin gene, plasma prothrombin concentration, and incidence of venous thromboembolism. Am J Hematol 2002;71:285-290.
37. Reynaud Q, Lega J, Mismetti P, Chapelle C, Wahl D, Cathébras P, et al. Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: A systematic review and meta-analysis. Autoimmunity Reviews 2014;13:595-608.
38. van Walraven C, Jennings A, Oake N, Fergusson D, Forster AJ. Effect of study setting on anticoagulation control: a systematic review and metaregression. Chest 2006;129:1155-1166.
39. Luna-Zaizar H, Gonzalez-Moncada AI, Padilla-Lopez EL, Ramirez-Anguiano AC, Pacheco-Moises FP, Velasco-Ramirez SF, et al. Thrombin generation and international normalized ratio in inherited thrombophilia patients receiving thromboprophylactic therapy. Thromb Res 2015;136:1291-1298.
40. Hemker HC, Al Dieri R, De Smedt E, Beguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost 2006;96:553-561.
41. Al Dieri R, de Laat B, Hemker HC. Thrombin generation: what have we learned? Blood Rev 2012;26:197-203.
42. Dargaud Y, Beguin S, Lienhart A, Al Dieri R, Trzeciak C, Bordet JC, et al. Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. Thromb Haemost 2005;93:475-480.
43. Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. JAMA 2006;296:397-402.
44. Gurwitz JH, Avorn J, Ross-Degnan D, Choodnovskiy I, Ansell J. Aging and the anticoagulant response to warfarin therapy. Ann Intern Med 1992;116:901-904.

45. Shepherd AM, Hewick DS, Moreland TA, Stevenson IH. Age as a determinant of sensitivity to warfarin. Br J Clin Pharmacol 1977;4:315-320.
46. Mueller JA, Patel T, Halawa A, Dumitrascu A, Dawson NL. Warfarin dosing and body mass index. Ann Pharmacother 2014;48:584-588.
47. Ring A, Schall R, Loke YK, Day S. Statistical reporting of clinical pharmacology research. Br J Clin Pharmacol 2017;83:1159-1162.
48. Moll S. New insights into treatment of venous thromboembolism. Hematology Am Soc Hematol Educ Program 2014;2014:297-305.

Table 1: Characteristics of the 50 patients

Age, years, mean (range)	47 (20-76)
Women (%)	34 (68)
Height, cm, mean (SD)	168 (11)
Weight, kg, mean (SD)	75 (19)
BMI, mean (SD)	27 (6)
*CYP2C9*1/*2*, n (%)	8 (16)
*CYP2C9*1/*3*, n (%)	7 (14)
*CYP2C9*2/*3*, n (%)	5 (10)
VKORC1 c.-1639G/A genotype, n (%)	17 (34)
VKORC1 c.-1639A/A genotype, n (%)	10 (20)
ASA use, n (%)	11 (22)
Statin use, n (%)	10 (20)
Smoking, n (%)	4 (8)

BMI, body mass index; *CYP2C9*, cytochrome P2C9 gene; *VKORC1*, vitamin K epoxide reductase complex 1 gene, ASA, acetylsalicylic acid

Table 2: Warfarin indications, and corresponding predicted warfarin dose estimates and real-life stable dose according to the specific indication, n=50

	n (%)	warfarindosing.org estimate, mg/d (SD)	IWPC estimate, mg/d (SD)	Real-life dose, mg/d (SD)
DVT or PE	33 (66)	5.7 (2.1)	5.4 (1.9)	7.4 (5.0)
Peripheral arterial thrombosis	4 (8)	3.2 (0.9)	3.2 (1.0)	3.9 (2.9)
Atrial fibrillation and ischemic stroke	2 (4)	3.0 (1.1)	2.8 (1.6)	2.5 (2.0)
Other ischemic stroke	2 (4)	5.6 (1.7)	5.7 (1.8)	7.7 (3.7)
Other thrombosis†	5 (10)	6.1 (1.2)	6.2 (0.8)	7.0 (2.9)
Valvular replacement and other comorbidity	4 (8)	5.9 (1.7)	5.7 (1.3)	7.4 (3.7)

† Other thrombosis included paroxysmal nocturnal hemoglobinuria, thrombi of vena cava, of sinus cavernosus, of portal and hepatic vein; DVT, deep vein thrombosis; IWPC, International Warfarin Pharmacogenetics Consortium; PE, pulmonary embolism; SD, standard deviation

Table 3: Thrombophilias detected in the laboratory screen, n=50

Thrombophilia	n (%)
FV Leiden heterozygous	10 (20)
FV Leiden homozygous	2 (4)
FII variant heterozygous	4 (8)
FV Leiden and *FII variant*, both heterozygous	2 (4)
Antiphospholipid antibodies	7 (14)
AT, PC or PS deficiency	4 (8)
Elevated FVIII:C	6 (12)
No thrombophilia in screening	21 (42)
Thrombophilia screen not done (valvular cases)	3 (6)

AT, antithrombin; PC, protein C; PS, protein S; FVIII:C, FVIII measured with clotting assay

Table 4: Distribution of warfarin genotypes

		VKORC1			
		c.-1639G/G	**c.-1639G/A**	**c.-1639A/A**	**Total**
CYP2C9	****1/*1***	14	12	4	30
	****1/*2***	4	1	3	8
	****1/*3***	3	3	1	7
	****2/*3***	2	1	2	5
Total		23	17	10	50

CYP2C9, cytochrome P2C9 gene, *VKORC1*, vitamin K epoxide reductase complex 1 gene

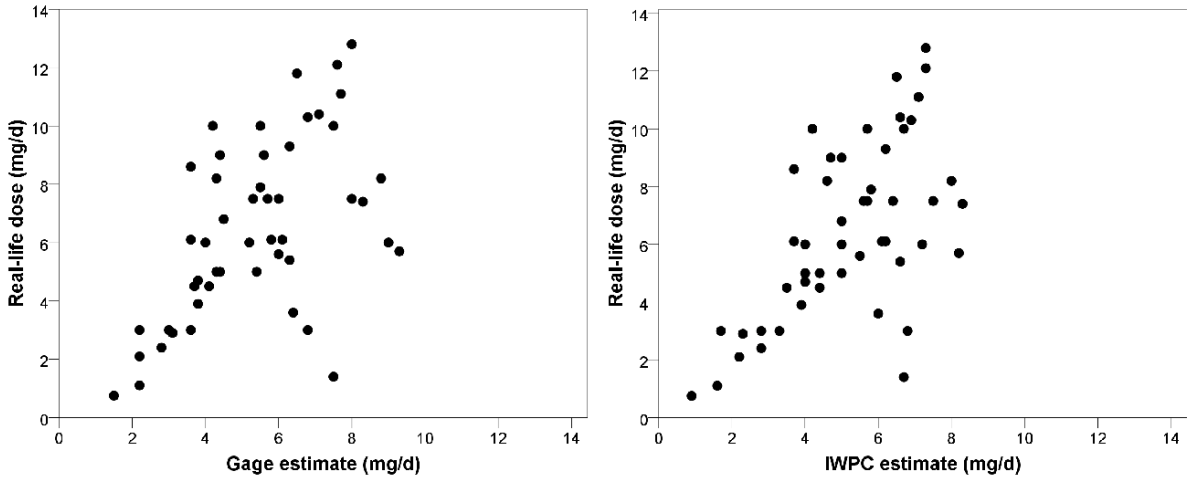

Figure 1: The correlation with the real-life dose was poor with both dose estimates: Gage estimated dose, R^2=0.26; IWPC estimated dose, R^2=0.29. The highest daily real-life dose of 30 mg is not visible in the figure. IWPC, International Warfarin Pharmacogenetics Consortium

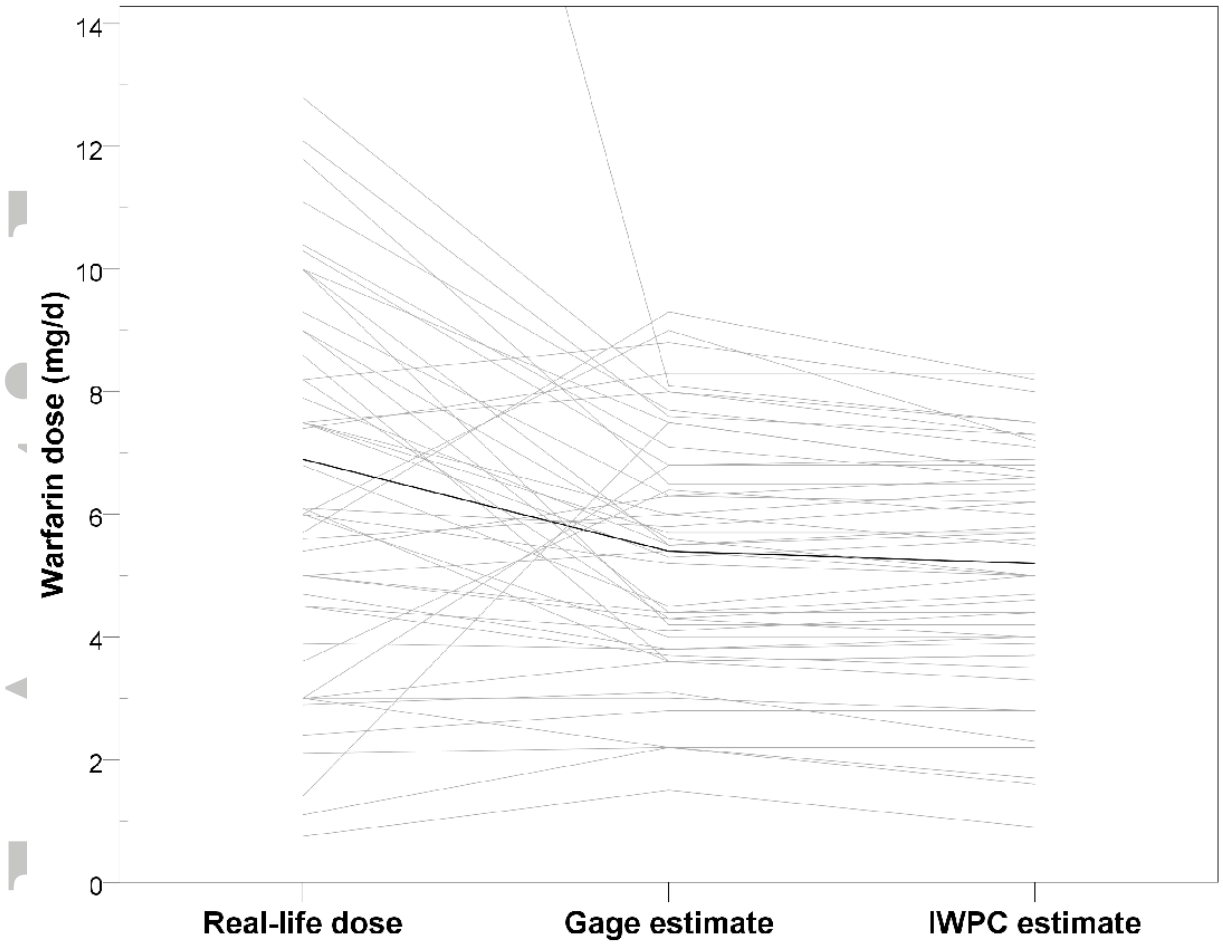

Figure 2: Mean daily real-life-dose was 6.9 mg, with Gage estimated dose 5.4 mg and IWPC estimated dose 5.2 mg. Real-life dose was higher than the dose estimates ($p<0.006$). Average dose (bold line) and individual patient doses are shown. The highest daily real-life dose of 30 mg is not visible in the figure. IWPC, International Warfarin Pharmacogenetics Consortium