

한국인 심방세동 환자에서 Warfarin 혈중 농도 측정의 임상적 유용성

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Determination of Plasma Warfarin Concentrations in Korean Patients and Its Potential for Clinical Application

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Background : Warfarin is a widely used oral anticoagulant with broad within- and between-individual dose requirements. Warfarin concentrations can be monitored by assessing its pharmacologic effects on International Normalized Ratio (INR). However, this approach has not been applied in the routine clinical management of patients receiving warfarin therapy. We performed a plasma warfarin assay using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) to determine if such an assay can be utilized in routine clinical practice.

Methods : We included a total of 105 patients with atrial fibrillation, and who were receiving warfarin for more than 1 yr. The plasma concentrations of total warfarin and 7-hydroxywarfarin were determined by HPLC-MS/MS (Waters, UK). We assessed the association between warfarin dose, concentration, and INR as well as the effects of these factors on warfarin concentrations.

Results : The mean maintenance dose of warfarin in 105 patients was 4.1 ± 1.3 mg/day (range, 1.7-8.0 mg/day) and their mean plasma warfarin concentration was 1.3 ± 0.5 mg/L. We defined a concentration range of 0.6-2.6 mg/L (corresponding to the 2.5th to 97.5th percentile range of the Plasma warfarin levels in the 74 patients showing INR within target range) as the therapeutic range for warfarin. The correlation of warfarin dose with warfarin concentration ($r^2=0.259$, $P<0.001$) was higher than that with INR ($r^2=0.029$, $P=0.072$).

Conclusions : There was a significant correlation between warfarin dose and plasma warfarin concentrations in Korean patients with atrial fibrillation. Hence, plasma warfarin monitoring can help determine dose adjustments and improve our understanding of individual patient response to warfarin treatment. (*Korean J Lab Med 2009;29:515-23*)

Key Words : Warfarin, Concentration, INR, Dose, Korean

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INTRODUCTION

Warfarin is widely used as an oral anticoagulant for the treatment of conditions associated with high risk of thromboembolic events such as atrial fibrillation, deep vein thrombosis, mechanical heart valve replacement, and pulmonary embolism [1, 2]. However, warfarin has a narrow therapeutic index and may be associated with adverse events. Inadequate or excessive anticoagulation may result in substantial morbidity and mortality due to thromboembolic complications or bleeding [3, 4]. Life-threatening bleeding episodes after initiation of warfarin therapy have been reported in approximately 12% of patients; furthermore, elderly patients are especially vulnerable to such events, and there is also a substantial risk of developing warfarin resistance [5–9]. During maintenance therapy, only 50–60% of patients can be expected to achieve their target INR range [10, 11]. Therefore, much effort has to be directed toward monitoring the safety and efficacy of warfarin treatment and developing strategies to predict the patient's response to this drug [12].

There is a marked within- and between-individual variability in warfarin dose requirements and different factors associated with patient response to warfarin treatment, which makes the monitoring of warfarin therapy more difficult [13–19]. With regard to the ethnic differences among patients, it has been shown that Asian populations require a markedly lower warfarin maintenance dose than Caucasians and African-Americans; however, the mechanisms underlying these differences still remain elusive [20, 21].

Currently, the required dose of warfarin is monitored based on its pharmacodynamic effects on the blood prothrombin time (PT) by using the standard international normalized ratio (INR) [22]. The assessment of plasma warfarin concentration may be valuable in some clinical situations; for example, to investigate unexpected changes in INR values in cases with long-term maintenance, as well as in cases in which therapeutic INR was not achieved after the initiation of standard warfarin treatment. Confirmation of the plasma warfarin concentration can direct further patient management. However, warfarin levels are

not routinely monitored in the clinical management of patients receiving warfarin therapy, in part because of the absence of readily available warfarin assays.

We developed a rapid plasma warfarin assay that involved evaluation of warfarin concentrations by using high-performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS). We assessed the relation between warfarin dose, plasma warfarin concentration, and INR, as well as the effects of these factors on the warfarin concentrations to determine if a warfarin assay could be utilized in routine clinical practice.

MATERIALS AND METHODS

1. Study subjects

The Institutional Review Board approved the study protocol. Written informed consent was obtained from each participant before participation in the study. One hundred and five Korean patients on stable warfarin therapy were recruited from a cardiovascular clinic. We included adult patients with atrial fibrillation who were receiving warfarin for at least 1 yr. Stable warfarin therapy was defined by a warfarin dose with constant INRs within the target range (2.0–3.0) over at least 3 consecutive monthly clinic visits just before the index visit. Demographics and clinical information including gender, age, body weight, medical history, medication profile, alcohol consumption, and smoking status were collected from patient medical records and questionnaires. Routine biochemical tests including liver function tests, lipid profile and renal function tests, as well as PT and plasma warfarin measurement for eligible patients were performed at the index visit. For the plasma warfarin assay, a single blood sample was obtained at least 12 hr after the administration of the last dose of warfarin.

2. Determination of warfarin and 7-hydroxywarfarin concentrations in plasma

The plasma concentrations of total warfarin and 7-hy-

droxywarfarin were determined by HPLC-MS/MS. Analyses were performed on a Quattro Micro API tandem mass spectrometer (Waters, Manchester, UK) equipped with a Waters 2795 Alliance HPLC system. The column used was a 3- μ m Atlantis[®] dC18 (2.1 \times 30 mm). The mobile phases consisted of aqueous solutions of 0.2 mM ammonium acetate and 0.1% formic acid and 40% acetonitrile. After simple protein precipitation with ZnSO₄, the Plasma samples were mixed with an internal standard (I.S.; chlorowarfarin) and centrifuged for 3 min. Quantitative analysis was performed in the multiple reaction-monitoring mode (m/z 307.0 \rightarrow 160.9 for warfarin; 322.9 \rightarrow 176.8 for 7-hydroxywarfarin; and 340.9 \rightarrow 283.8 for I.S.) with a total running time of 150 s for each sample (Fig. 1). The linear assay range was 75–7,500 ng/mL for both total warfarin and 7-hydroxywarfarin ($r^2 > 0.99$). The intra- and inter-assay imprecision measures were CV 1.2–2.5% and CV 0.7–7.2% for total warfarin and CV 2.1–7.2% and CV 0.5–7.0% for 7-hydroxywarfarin, respectively.

3. Statistical analysis

Continuous variables were reported as mean \pm SD, medians, and ranges. The distribution of quantitative variables was tested for normality by a one-sample Kolmogorov-

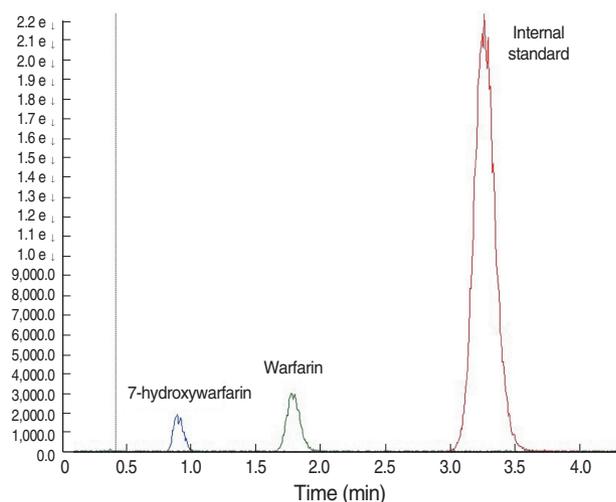


Fig. 1. Chromatograms of 7-hydroxywarfarin (m/z 322.9 \rightarrow 176.8), warfarin (m/z 307.0 \rightarrow 160.9), and internal standard (chlorowarfarin; m/z 340.9 \rightarrow 283.8).

Smirnov test. The differences between the groups categorized by gender, alcohol, and smoking habit were analyzed by the Wilcoxon two-sample test. The associations between plasma warfarin concentrations and warfarin dose or INR were evaluated by the Spearman correlation test. The increasing age trend and warfarin concentration were evaluated by the Jonckheere-Terpstra trend test. A multiple linear regression analysis was used to model the relationships of plasma warfarin concentration with other variables. A P value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SAS software (version 9.1.3; SAS, Cary, NC, USA).

RESULTS

One hundred and five patients participated in the present study. Table 1 summarizes their profiles including clinical conditions, warfarin dose, and plasma warfarin concentrations. Ninety-six (91%) of the patients were receiving

Table 1. Characteristics of patients with atrial fibrillation (N=105)

Demographics	
Age (yr)	67.8 \pm 9.8 (36-87)
Gender (male:female)	66:39
Body weight (kg)	65.6 \pm 11.2 (39.0-96.0)
Body surface area (m ²)	1.70 \pm 0.19 (1.22-2.09)
Coexisting disease condition (%)	73 (69)
Concurrent medications (%)	96 (91)
Warfarin therapy	
Duration (yr)	3.2 \pm 2.2 (1-8)
Warfarin dose (mg/day)	4.1 \pm 1.3 (1.7-8.0)
Warfarin dose (mg/kg/day)	0.45 \pm 0.15 (0.16-1.06)
Plasma drug concentrations	
Total warfarin (mg/L)	1,343.4 \pm 522.1 (420.2-3,310.3)
7-hydroxywarfarin (mg/L)	90.9 \pm 55.3 (16.0-344.4)
Warfarin/7-hydroxywarfarin ratio	17.7 \pm 8.8 (6.3-57.8)
PT INR	2.31 \pm 0.44 (1.42-3.94)
Other laboratory findings	
Albumin (g/dL)	4.1 \pm 0.3 (2.9-4.9)
Aspartate aminotransferase (U/L)	25.8 \pm 8.5 (13-63)
Alanine aminotransferase (U/L)	22.5 \pm 11.1 (5-71)
Total bilirubin (mg/dL)	0.8 \pm 0.4 (0.2-2.0)
Creatinine (mg/dL)	1.1 \pm 0.3 (0.60-2.65)
Total cholesterol (mg/dL)	168.0 \pm 27.6 (108-221)
Triglyceride (mg/dL)	128.9 \pm 56.6 (47-302)
Low-density lipoprotein cholesterol (mg/dL)	109.4 \pm 25.6 (49-159)

Values represented as mean \pm SD (range).

Abbreviation: PT INR, prothrombin time international normalized ratio.

concurrent medications, including antiarrhythmics (N=43), diuretics (N=31), aspirin (N=16), antilipemic agents (N=9), oral diabetic agents (N=6), allopurinol (N=4), beta-adrenergic blockers (N=2), steroids (N=2), antidepressants (N=2), nonsteroidal anti-inflammatory drugs (NSAIDs) (N=2), and antihistamines (N=1). Twenty-six patients were using drugs that could potentially increase their INR (aspirin, simvastatin, allopurinol, or NSAIDs) [12, 23, 24]. Over 69% of the patients had underlying comorbid conditions, including diabetes mellitus (N=19), hypertension (N=17), valvular heart disease (N=10), asthma (N=5), lung disease (N=5), thyroid disease (N=3), arthritis (N=3), chronic hepatitis (N=2), and other diseases (N=9). In addition, 49.5% (52/105) of patients experienced a variety of bleeding complications, including epistaxis, gum bleeding, melena, and hematuria; however, no patient experienced any serious bleeding episode, and 5.7% (6/105) of patients experienced thrombotic complica-

tions during warfarin treatment, including cerebral infarction and mesenteric infarction.

The correlations among plasma warfarin concentrations, warfarin dose requirements, and INR were studied in the 105 patients. There was no significant correlation between INR and plasma warfarin concentration or weekly warfarin dose, as shown in Fig. 2A, B. However, a significant correlation was found between plasma warfarin concentration and weekly warfarin dose ($r^2=0.259$, $P<0.001$) (Fig. 2C). The body weight-adjusted warfarin doses exhibited strong correlation with warfarin concentrations ($r^2=0.414$, $P<0.001$) (Fig. 2D). At the time of warfarin measurement, 74 out of 105 patients had INR values between 2.0 and 3.0. The warfarin dose, total plasma warfarin concentration, and warfarin to 7-hydroxywarfarin ratio for the 74 patients who were well controlled are presented in Fig. 3. The mean warfarin maintenance dose was 4.1 mg/day.

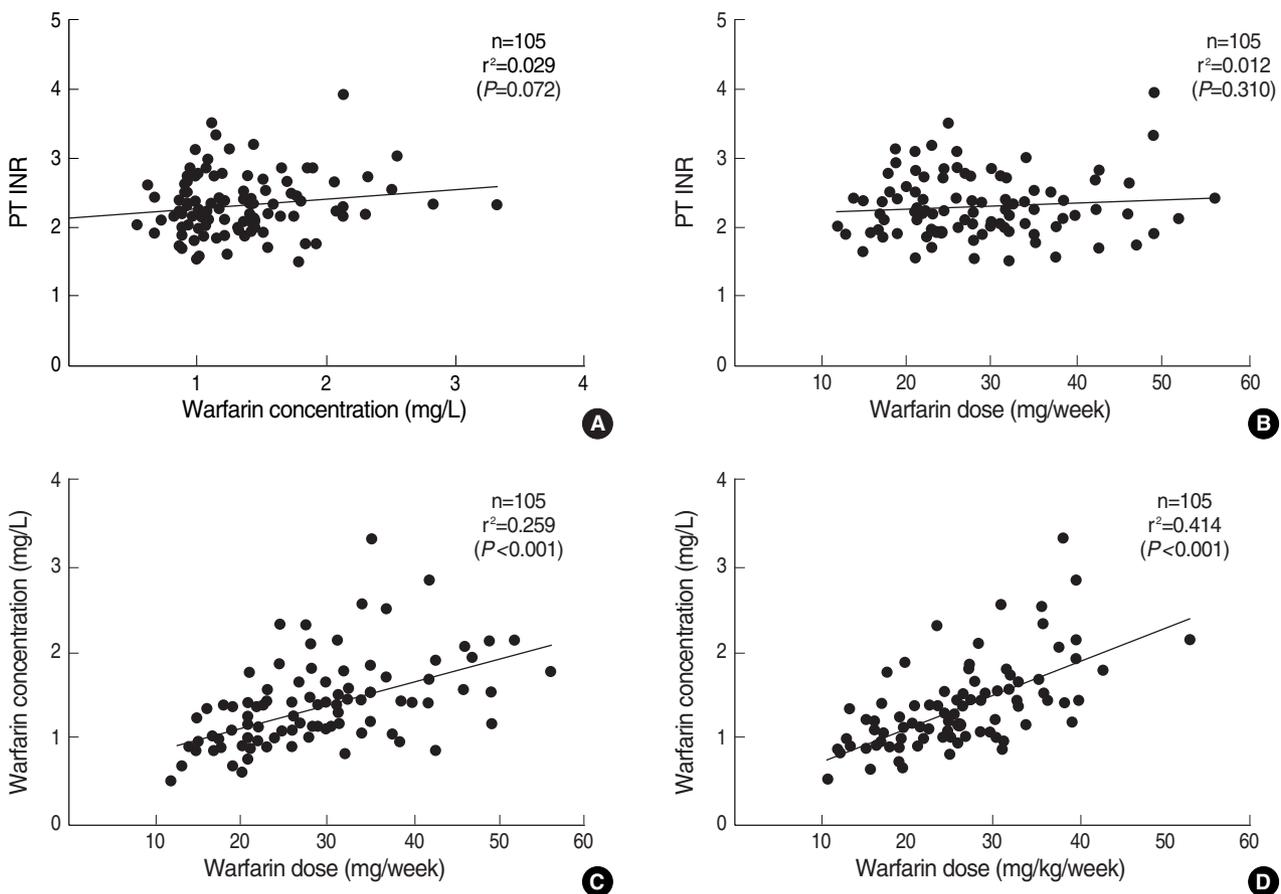


Fig. 2. Correlation of PT INR, plasma warfarin concentration, and warfarin dose. Abbreviation: PT INR, prothrombin time international normalized ratio.

Warfarin dose requirements showed a wide inter-patient variation, i.e., 1.7–8.0 mg/day (4.7-fold range) and 0.03–0.13 mg/kg/day (4.9-fold range). The warfarin concentrations and ratios also showed significant inter-patient variation, i.e., 0.4–3.3 mg/L (7.7-fold range) and 6.3–57.8 mg/kg/day (9.2-fold range), respectively. The plasma warfarin concentration was not normally distributed. The 2.5th and 97.5th percentiles were used to set the therapeutic range for 74 patients, who showed a target INR in the range of 2.0–3.0 at the time of warfarin measurement; the range was finally set to a 0.6–2.6 mg/L interval.

Warfarin concentration in men (1.2 vs 1.5 mg/L, $P=0.005$) was lower than that in women (4.2 vs 3.9 mg/day, $P=0.181$) at similar doses. The warfarin concentrations did not vary significantly with age ($P=0.445$). Warfarin concentrations (mg/L) in the following age groups—age <50 yr, 51–60 yr,

61–70 yr, 71–80 yr, and ≥ 80 yr were as follows: 1.3 ± 0.2 , 1.2 ± 0.4 , 1.4 ± 0.5 , 1.3 ± 0.5 , and 1.4 ± 0.6 mg/L, respectively. However, the warfarin dose (mg/day) declined significantly with increasing age as follows: 6.0 ± 1.0 , 4.2 ± 1.4 , 4.3 ± 1.4 , 3.8 ± 1.2 , and 3.6 ± 0.8 ($P=0.004$). Plasma albumin concentrations showed a positive but weak correlation with warfarin concentrations ($r^2=0.127$, $P=0.001$). Smoking and alcohol dependence did not show any significant effect on warfarin concentration and daily dose. The warfarin concentrations were log-transformed to determine a normal distribution, thus allowing parametric tests and regression analysis to be performed. Stepwise regression was used to evaluate the association between warfarin concentration and other variables. Although we found that warfarin dose ($r^2=0.29$, $P<0.001$) significantly affected plasma warfarin concentration, plasma albumin ($r^2=0.04$, $P=0.012$) and con-

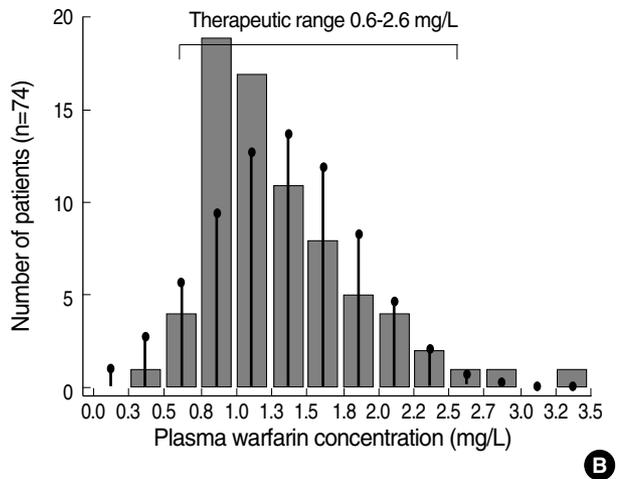
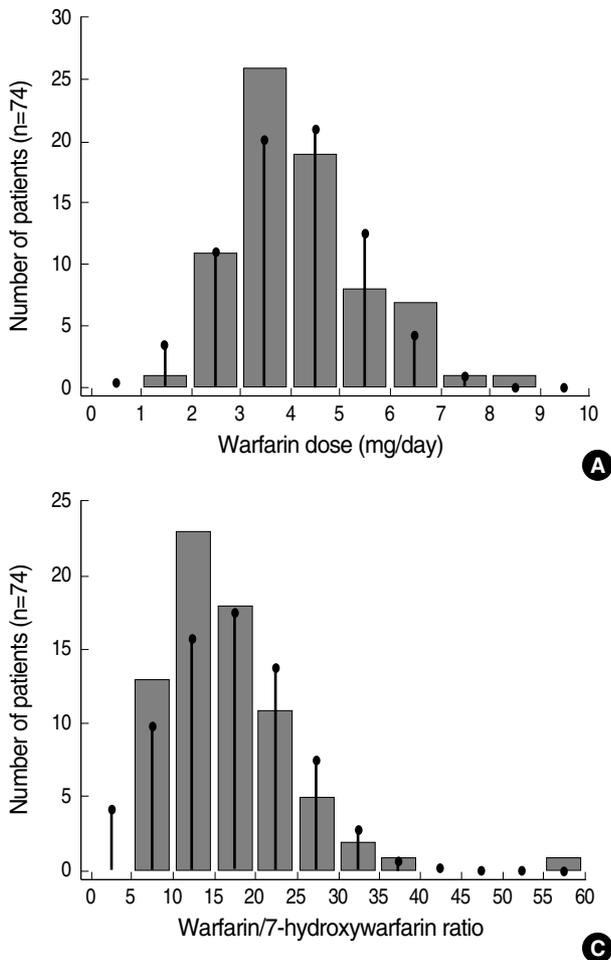


Fig. 3. (A) The distributions of warfarin dose in 74 patients with PT INR within the target range of 2.0–3.0. (B) The total warfarin concentrations and the middle 95th percentile interval (0.6–2.6 mg/L) in this group. (C) The plasma warfarin to 7-hydroxywarfarin concentration ratio in this group. The vertical lines indicate the extent of normal distribution of the data.

current medications ($r^2=0.002$, $P=0.023$) did not greatly contribute to the variation.

DISCUSSION

Warfarin has a narrow therapeutic index and may be associated with severe adverse events such as bleeding complications. Due to the variation in drug response, the use of the same fixed initial dose or maintenance dose of warfarin for all patients is unachievable and undesirable. Therefore, warfarin therapy requires close monitoring and dosing adjustment for every individual. PT measurements appear to be feasible for routine monitoring of the patient warfarin response, although the relationship between warfarin dose and PT response is poor. The warfarin assay has rarely been applied in clinical practice, and there have been very few reports on the determination of plasma warfarin concentrations using this assay. This dearth of studies can be attributed to the inconclusive data on the concentration-response relationship of warfarin and the unavailability of a warfarin assay for routine analysis. However, the plasma warfarin assay has potential value in the assessment of cases with an unusual response to warfarin. Such an assay may be used to distinguish between cases with an abnormal concentration-response relationship (pharmacodynamics) and those with an unusual dose-concentration relationship (pharmacokinetics).

We assessed the correlation between warfarin concentration, dose, INR, and other variables. We observed that wide variations of warfarin dose (4.7-fold) and plasma warfarin concentration (7.7-fold) were required to reach the therapeutic INR range. The results of this study confirmed that Koreans have a lower warfarin dose requirement than Caucasians and African-Americans. To achieve a target INR of 2.0–3.0, Caucasians and African-Americans require warfarin doses of 4.7 ± 2.4 mg/day and 5.3 ± 2.6 mg/day, respectively [25]. However, the maintenance dose of warfarin for the Korean patients in our study (4.1 ± 1.3 mg/day) was lower than the doses for Caucasians and African-Americans. This variation in different populations may be attributed to the different dietary habits as well as genet-

ic factors. Recently, *VKORC1* and *CYP2C9* polymorphisms have been suggested to contribute to the differences in the responses of different ethnic groups [13–19].

The therapeutic range is the range of drug levels within which the probability of the desired clinical response is relatively high and that of unacceptable toxicity is relatively low. The therapeutic range for warfarin is difficult to establish because of the less clear concentration-effect relationship. The previously reported ranges vary from 0.5–3 mg/L to 1–10 mg/L [23]. We defined a concentration range of 0.6–2.6 mg/L (corresponding to the 2.5th to 97.5th percentile range of the Plasma warfarin levels in the 74 patients showing INR within target range) as the therapeutic range of warfarin.

We assessed the correlations among the plasma warfarin concentrations, warfarin dose requirements, and INR. Although the INR has been widely accepted as the standard parameter for monitoring of response to oral anticoagulation therapy, we found a poor correlation between warfarin dose and INR, and this finding was consistent with the findings reported in the previous studies [26–28]. We found that plasma warfarin concentration, rather than INR, was more closely associated with warfarin dose requirement.

The response to warfarin is influenced by many pharmacokinetic and pharmacodynamic factors. Thus, the determination of INR alone cannot be adequate for dosage adjustment in complicated clinical environments. The influence of a variety of additional factors must be taken into account to understand the anticoagulation effects. When the warfarin concentration is not low and the INR is not within the target range, factors other than the warfarin dose should be taken into account to determine the cause of warfarin resistance. Furthermore, confirmation of the plasma warfarin concentration after the initiation of standard warfarin treatment is helpful in patients who show an unexplained increase in INR, or in cases in which the INR target range is not achieved. In addition, the plasma warfarin concentration can be used as an indicator of patient compliance or drug resistance. Serial measurements of plasma warfarin concentration may help in the identi-

fication of other factors influencing patient sensitivity to warfarin as well as in the adjustment of warfarin dose to more objective and specific markers. This approach would be especially useful in patients showing difficulties in maintaining the target INR or in those who are noncompliant toward warfarin treatment. Further studies are needed to identify the mechanisms underlying the poor correlation between warfarin concentration and INR for the patients in whom the target INR is not achieved even under therapeutic plasma warfarin concentrations.

Genetic variation in cytochrome P-450 2C9 (*CYP2C9*) is responsible for the metabolic clearance of the more pharmacologically potent (*S*)-warfarin enantiomer; further, vitamin K epoxide reductase complex subtype 1 (*VKORC1*) is at least partly responsible for warfarin resistance, which causes significant differences in patient responses to warfarin therapy [29, 30]. In our study, 2 patients receiving 6 mg/day and 5 mg/day warfarin showed high plasma warfarin concentrations of 2,825 mg/L and 3,310 mg/L, respectively; these plasma warfarin concentrations corresponded to INR values of up to 2.29 and 2.16, respectively. Although their daily warfarin dose requirements were not very high, the body weight-adjusted doses of these patients were high, belonging to the upper 5% of the dose spectrum. Further investigation is required to fully characterize the warfarin resistance of the pharmacogenetic determinants such as the *VKORC1* genotype. We performed direct sequencing of the *VKORC1* gene with the consent of the 2 patients, and detected a 1173 C/T polymorphism.

Several HPLC methods have been introduced to determine warfarin concentrations [31–35]; however, none of these have been found to be suitable for routine use in the clinical setting. Our HPLC–MS/MS method for the measurement of warfarin and its metabolites has several advantages over the previously suggested methods that are time consuming. With its high speed and analytical accuracy, our technique can be readily incorporated into routine operations in the clinical setting and would be helpful to assess warfarin response.

At present, the standard clinical practice is to adjust warfarin dose based on PT and clinical response. However, these

approaches do not provide accurate assessment of warfarin therapy. Patient management with warfarin therapy is still difficult. Additional plasma warfarin monitoring can serve as an important guide for dose adjustments and improve our understanding of individual patient response to warfarin treatment.

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