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Original Investigation

# Influence of Kidney Function on Risk of Supratherapeutic International Normalized Ratio–Related Hemorrhage in Warfarin Users: A Prospective Cohort Study

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**Background:** Anticoagulation management is difficult in chronic kidney disease, with frequent supratherapeutic international normalized ratios (INRs $\geq 4$) increasing hemorrhagic risk. We evaluated whether the interaction of INR and lower estimated glomerular filtration rate (eGFR) increases hemorrhage risk and whether patients with lower eGFRs experience slower anticoagulation reversal.

**Study Design:** Prospective cohort study.

**Setting & Participants:** Warfarin pharmacogenetics cohort (1,273 long-term warfarin users); warfarin reversal cohort (74 warfarin users admitted with INRs $\geq 4$).

**Predictor:** eGFR, INR as time-dependent covariate, and their interaction in the pharmacogenetics cohort; eGFR in the reversal cohort.

**Outcomes & Measurements:** In the pharmacogenetics cohort, hemorrhagic (serious, life-threatening, and fatal bleeding) risk was assessed using proportional hazards regression. In the reversal cohort, anticoagulation reversal was assessed from changes in INR, warfarin and metabolite concentrations, clotting factors (II, VII, IX, and X), and PIVKA-II (protein induced by vitamin K absence or antagonist II) levels at presentation and after reversal, using linear regression and path analysis.

**Results:** In the pharmacogenetics cohort, 454 (35.7%) had eGFRs $< 60$ mL/min/1.73 m$^2$. There were 137 hemorrhages in 119 patients over 1,802 person-years of follow-up (incidence rate, 7.6 [95% CI, 6.4-8.9]/100 person-years). Patients with lower eGFRs had a higher frequency of INR $\geq 4$ ($P < 0.001$). Risk of hemorrhage was affected significantly by eGFR-INR interaction. At INR $< 4$, there was no difference in hemorrhage risk by eGFR (all $P \geq 0.4$). At INR $\geq 4$, patients with eGFRs of 30 to 44 and $<30$ mL/min/1.73 m$^2$ had 2.2-fold (95% CI, 0.8-6.1; $P = 0.1$) and 5.8-fold (95% CI, 2.9-11.4; $P < 0.001$) higher hemorrhage risks, respectively, versus those with eGFRs $\geq 60$ mL/min/1.73 m$^2$. In the reversal cohort, 35 (47%) had eGFRs $< 45$ mL/min/1.73 m$^2$. Patients with eGFRs $< 45$ mL/min/1.73 m$^2$ experienced slower anticoagulation reversal as assessed by INR ($P = 0.04$) and PIVKA-II level ($P = 0.008$) than those with eGFRs $\geq 45$ mL/min/1.73 m$^2$.

**Limitations:** Limited sample size in the reversal cohort, unavailability of antibiotic use and urine albumin data.

**Conclusions:** Patients with lower eGFRs have differentially higher hemorrhage risk at INR $\geq 4$. Moreover, because the INR reversal rate is slower, hemorrhage risk is prolonged.

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***INDEX WORDS:*** Kidney function; chronic kidney disease (CKD); warfarin; supra-therapeutic international normalized ratio (INR); pharmacokinetics; hemorrhage; reversal of anticoagulation; adverse event.

Therapy with warfarin, the most commonly prescribed oral anticoagulant, is challenging because of the many factors that influence its pharmacokinetics and pharmacodynamics.[1] Despite concerted efforts, anticoagulation management remains suboptimal, with frequent supratherapeutic international normalized ratios (INRs) often associated with hemorrhagic complications.[2,3] This reality has earned warfarin a consistent ranking among the top 10 drugs associated with serious adverse events.[4]

There has been growing appreciation that decreased kidney function affects the clearance of (and response to) drugs that are metabolized mainly by the liver, such as warfarin.[5-7] Although anticoagulation management among patients with chronic kidney disease (CKD) is particularly challenging, initiation and management of warfarin therapy in patients with CKD are similar to those in the general medical population.[8,9] We previously have reported that patients with CKD require lower warfarin doses to maintain a therapeutic

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INR, have worse anticoagulation control, and have a higher risk of hemorrhage compared with patients with normal kidney function.[10-12]

The goal of the present study was to evaluate whether patients with CKD have a differentially higher risk of hemorrhage during episodes of supratherapeutic INR (INR $\geq 4$) in the warfarin pharmacogenetics cohort, and whether decreased kidney function influences the rate of INR reversal among patients with episodes of supratherapeutic INR in the warfarin reversal cohort. Finally, we provide preliminary data on a potential mechanism by which decreased kidney function influences supratherapeutic INR, facilitated by assessment of PIVKA-II (protein induced by vitamin K absence or antagonist II) in the warfarin reversal cohort.

## METHODS

### Patient Characteristics and Study Design

The warfarin pharmacogenetics cohort (institutional review board protocol numbers X030102003 [Pharmacogenetic Optimization of Anticoagulation Therapy] and X080114012 [Genetic and Environmental Determinants of Warfarin]) recruited patients 20 years or older initiating warfarin therapy with a target INR range of 2 to 3. The aims of the study were to identify the influence of clinical and genetic factors on warfarin dose and hemorrhage. These data supported evaluating the interaction of kidney function and supratherapeutic INR (INR $\geq 4$) on risk of hemorrhage.

A detailed history documented information including race, demographics, height and weight, indication for warfarin therapy, comorbid conditions, medications, and socioeconomic factors, in addition to laboratory values (serum urea nitrogen, serum creatinine, hemoglobin, and hematocrit) as detailed in recent publications. Genotyping methodology for the cytochrome P450 (CYP) genes *CYP2C9* and *CYP4F2* and the gene encoding vitamin K oxidoreductase complex subunit 1 (*VKORC1*) has been reported previously.[10,13,14] All patients were followed up at least monthly[8] for up to 2 years from initiation of therapy (or for the duration of therapy if <2 years). Variables influencing warfarin response, such as warfarin dose, INR, concurrent medications (such as statins, antiplatelet agents, and amiodarone), dietary vitamin K and alcohol intake, and medication adherence were recorded at each visit.

Patients using warfarin with supratherapeutic INRs reported on admission were identified. The treating physicians were contacted and patients were enrolled in the warfarin reversal cohort (institutional review board protocol number X090911007) if they were to receive vitamin K to reverse the INR. Warfarin users (n = 102; age $\geq 20$ years) hospitalized with supratherapeutic INRs (INR $\geq 4$; visit 1) were recruited prior to administration of vitamin K per guidelines.[8] A structured interview form was used at the time of enrollment to obtain a detailed medical lifestyle, social, and concomitant medication history as in the other cohort. Patients were followed up until INR had decreased by > 50% from the initial INR (visit 2). Patients who received plasma or clotting factors (due to medical necessity; n = 28) were excluded from the analysis. The other 74 patients were followed up until INR had decreased by > 50% from the initial INR (visit 2). Blood samples (DNA, plasma, and serum) were collected at both times. Single-nucleotide polymorphisms (SNPs) in *CYP2C9*, *VKORC1*, and γ-glutamyl carboxylase (*GGCX*; reference SNP identification number rs11676382) were assessed. This supported the assessment of influence of kidney function on anticoagulation reversal among warfarin users hospitalized with supratherapeutic INRs.

All plasma and serum samples were processed within 30 minutes of blood collection and archived at −70°C. For both visits 1 and 2, plasma samples were analyzed for vitamin K–dependent clotting factors (factors II, VII, IX, and X; University of Alabama at Birmingham Hospital laboratories) using the coagulation analyzer STAR (Stago). PIVKA-II was used to assess functional vitamin K status. The PIVKA-II assay was performed on plasma using a murine monoclonal antibody available in an enzyme immunoassay kit (Asserachrom PIVKA-II; Stago) at the Tufts University vitamin K laboratory as previously reported. Serum samples were analyzed to determine total warfarin and metabolite concentrations (see Item S1, available as online supplementary material) at the University of Pittsburgh.

### Assessment of Kidney Function

Kidney function was assessed using estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation.[15,16] Serum creatinine was determined by the Jaffé rate method standardized to isotope-dilution mass spectrometry. Patients were categorized into 4 groups based on eGFR: ≥60 (reference group), 45 to 59 (CKD stage 3a), 30 to 44 (CKD stage 3b), and <30 mL/min/1.73 $m^2$ (CKD stages 4 and 5). Patients receiving maintenance dialysis were categorized in the latter group. Both studies were conducted under the approval of the Institutional Review Board of the University of Alabama at Birmingham.

### Outcome Definitions and Statistical Analysis

Supratherapeutic INR was defined as an episode of INR $\geq 4$ among patients on warfarin therapy.[3,17] Major hemorrhages included serious, life-threatening, and fatal bleeding episodes.[18] For all hemorrhagic events, complication site (eg, gastrointestinal), gravity of the event (eg, requiring medical/surgical intervention), and laboratory findings at the time of the event were objectively documented. Isolated sub- or supratherapeutic INRs in the absence of evidence of bleeding were not classified as events. Minor hemorrhages (nosebleeds, microscopic hematuria, bruising, and mild hemorrhoidal bleeding) were not included.

During follow-up, all hemorrhagic complications were captured and verified through review of admissions and emergency department visits. Only medically documented events were included in the analyses. The Alabama Center for Health Statistics was queried to verify cause of death for all deceased patients to ensure inclusion of deaths due to hemorrhagic complications. All complications were reviewed and adjudicated by a blinded reviewer.

### Statistical Methods

To assess unadjusted between-group differences across eGFR categories in both cohorts, we performed analysis of variance models for continuous variables and $\chi^2$ tests for categorical variables. To determine whether proportions of INRs $\geq 4$ across the eGFR categories in the warfarin pharmacogenetics cohort were significantly different, we used generalized estimating equations with the autoregressive lag-1 covariance structure to account for multiple INR measurements from the same patient because the density of the INRs differs across patients during clinical care.

Incidence rate of hemorrhage and confidence intervals (CIs) were calculated using SAS, version 9.3 (SAS Institute Inc). After adjusting for age, race, sex, genotype, concomitant medications, clinical comorbid conditions, and INR at the time of the event, the interaction between kidney function and INR (eGFR-INR) was evaluated using multivariable Cox proportional hazards regression with the counting process format.[19] This allowed us to account for multiple events and account for the INRs as a time-dependent covariate. Departures from the proportional hazards assumption were assessed by evaluating interactions of the predictors and a function of survival time.

We calculated rates of changes per hour for INR, PIVKA-II, warfarin concentrations, and clotting factor levels (II, VII, IX,