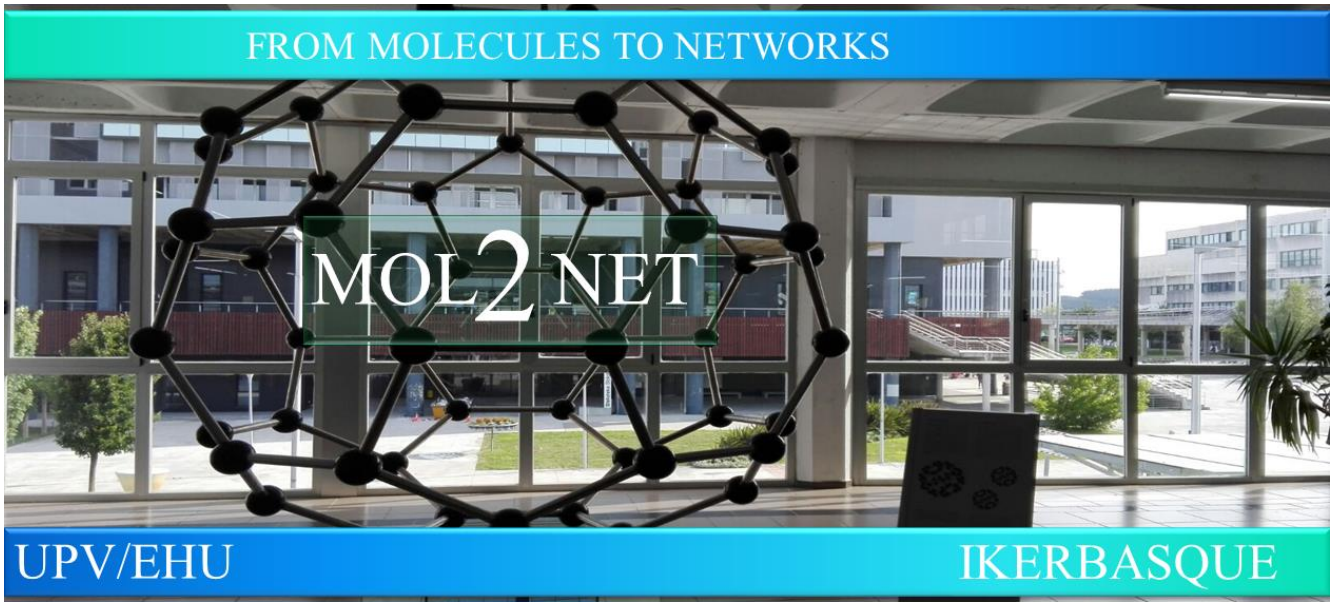
MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed.



Contribution of vitamin K and gut microbiota to individual variability of warfarin in cardiac surgery patients

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Abstract

Warfarin is a commonly prescribed anticoagulant in clinic. It has large individual variability. The pharmacokinetics and pharmacodynamics (PKPD) of warfarin had been established. There also exist some unknown factors that could affect warfarin effect. The purpose of the study was to explore the contribution of vitamin K and gut microbiota to individual variability of warfarin in cardiac surgery patients. A total of 246 patients were enrolled in the present study. Serum and fecal samples were collected used to detect warfarin and vitamin K (VK1 and MK4) concentrations and the diversity of gut microbiota, respectively. The demographic characteristics, drug history, and CYP2C9 and VKORC1 genotype were recorded from the patients' medical records. The INR and warfarin concentration bias were predicted according to the PKPD model. The results of INR and warfarin concentration predicted bias by the PKPD model were: 78.7% of Ideal prediction for INR, 66.7% of Ideal prediction for S-warfarin, 75.0% of Ideal prediction for R-warfarin. Only INR PE was compared with VK concentration and gut microbiota due to INR was the main indicator for warfarin therapy in clinic. The INR of patients decreased with increasing of VK concentration. The pharmacodynamic parameter C50 of S-warfarin, which was derived from the PKPD model, increased with increasing of VK concentration. The results diversity of gut microbiota showed that *Prevotella* might be associated with warfarin anticoagulation. In conclusion, vitamin K had some

effect on the individual variability of warfarin. The gut microbiota might also have a certain effect on the variability of warfarin.

Keywords

warfarin, vitamin K, gut microbiota

Introduction *(optional)*

Warfarin, as an oral anticoagulant, is widely prescribed worldwide (1-3). It has large individual variability and a narrow therapeutic index, and the daily dose variability may be up to 20-fold when achieving the target anticoagulant effect (4). Many factors contribute to the variability of warfarin. Numerous algorithms of warfarin have also been developed by pharmacokinetics and pharmacodynamics (PKPD) (5, 6). There also exist some unknown factors that could affect warfarin anticoagulants. The chemical structure of warfarin is similar to that of vitamin K. Warfarin exerts its anticoagulation by interfering with the synthesis of vitamin K dependent clotting factors via inhibition of vitamin K epoxide reductase complex 1 (VKORC1) (7). Vitamin K includes two major forms: phylloquinone (vitamin K1) and menaquinones (vitamin K2, MK4-MK13). Phylloquinone (vitamin K1) is found exclusively in plants, while menaquinone (MK5-MK13) is produced by a series of congeners synthesized by gram-positive bacteria in the human gastrointestinal tract (8, 9), MK4 is endogenously synthesized from phylloquinone in mammals (10) and is found in animal products. All forms of vitamin K have one well-known function and were identified as cofactors for the posttranslational enzyme γ-glutamate carboxylase, which is established by the common naphthoquinone ring structure. It is necessary for the generation of active coagulation factors II, VII, IX, and X (8, 9). The liver is the site of synthesis of vitamin K-dependent coagulation factors and was originally thought to be the major site of storage of vitamin K (10). Therefore, fluctuations in vitamin K concentration in the body may affect warfarin anticoagulant effects. Information on the physiological and pharmacological roles of vitamin K in vivo is still limited. One reason for this is that the detection and monitoring of vitamin K homologs in plasma and organs have been difficult on account of quite small concentrations and many kinds of impurities, even though measurement of the vitamin K plasma concentration is essential to optimize therapy (11). The detection of vitamin K1 has been developed in plasma, however, the detection of vitamin K2 is difficult in plasma. The human gut is thought to be a menaquinone reservoir and is estimated to contribute to 10–50% of the human vitamin K requirement (9, 12). Some studies indicated that phylloquinone accounted for less than 10% of the vitamin K content in the human liver (9). Vitamin K2 in vivo could be reflected by analyzing the gut microbiota composition. The gut microbiota is characterized by an interindividual variability due to genetic and environmental factors (13). The purpose of the present study was to explore the contribution of vitamin K and gut microbiota to individual variability of warfarin in cardiac surgery patients.

Materials and Methods *(optional)*

Patients and sampling

The present study was approved by the Health Authority Ethics Committee of the First Affiliated Hospital of Soochow University and was in accordance with the Declaration of Helsinki. All patients gave written informed consent. Patients underwent cardiac surgery for a variety of reasons, including valvular heart disease, rheumatic valve disease, infectious endocarditis, aortic dissection (Stanford A or B), ascending aortic aneurysm, congenital heart disease, *etc*. Routine detection of the international normalized ratio (INR) was performed in the hospital clinical laboratory. Approximately 2ml blood was drawn in a coagulation tube when drawing blood for INR detection. These blood samples were centrifuged, and then serum samples were restored at -80 ℃. The serum samples were used to detect S-warfarin, R-warfarin and vitamin K (VK1 and MK4) concentrations. CYP2C9 and VKORC1 were performed as part of standard clinical care.

Fecal samples of patients, who were preantibiotics, postantibiotics over 7 days and withdrawn antibiotics over 7 days, were also collected. All fecal samples were used to analyze the gut microbiota.

Bioanalysis

Total (bound plus unbound) serum concentrations of S-warfarin and R-warfarin were measured by liquid chromatography-mass spectrometry (LC-MS/MS) according to a previously established detection method (6). VK1 and MK4 concentrations were also measured by LC-MS/MS. The linear range for VK1 and MK4 was 0.05-5 ng/mL. The precision of the assay was within ±15%. The analysis of gut microbiota was detected by Majorbio (Shanghai) Co., Ltd.

Data analyzing

The INR and concentration bias were predicted according to the PKPD model previously established. The equation of predicted error (PE) as follow. High prediction was defined as PE lower than -20%, and low prediction was defined as PE higher than 20%. PE in the range of -20% to 20% was defined as an ideal prediction. Then, the contributions of vitamin K and gut microbiota to individual variability of warfarin were analyzed according to the INR PE.

$$\mathrm{PE} = \frac{INR_{observed} - INR_{prdicted}}{INR_{observed}} \times 100\%$$

Results and Discussion *(optional)*

Patient characteristics

A total of 246 patients, with 1469 observed warfarin concentrations, 3018 observed INRs, and 622 fecal samples, were enrolled in the present study. The demographic characteristics of the patients, indication for surgery, and drug history were recorded from the patients' medical records.

Predicted error by PKPD model

The results of INR and warfarin concentration predicted bias by the PKPD model are shown in Table 1.

Table 1. PE of INR and warfarin concentration by the PKPD model.

	High prediction	Ideal prediction	Low prediction
INR(n=3018)	415 (13.8%)	2374 (78.7%)	229 (7.6%)
S-warfarin(n=1469)	264 (18.0%)	980 (66.7%)	225 (15.3%)
R-warfarin(n=1469)	163 (11.1%)	1102 (75.0%)	204 (13.9%)

Contribution Vitamin K and gut microbiota to warfarin

Only INR PE was compared with VK concentration and gut microbiota, because INR was the main indicator for warfarin therapy in clinic. The relationship between the VK concentration and warfarin is shown in Figure 1. The INR of patients before warfarin treatment decreased with increasing of VK concentration (Figure 1A). The VK concentration of the patients was significantly reduced after warfarin treatment (Figure 1B). The pharmacodynamic parameter C50 of S-warfarin, which was derived from the PKPD model, increased with increasing of VK concentration (Figure 1C). The PE of INR increased with increasing VK concentration (Figure 1D). These results indicated that the VK concentration had a significant effect on warfarin variability.

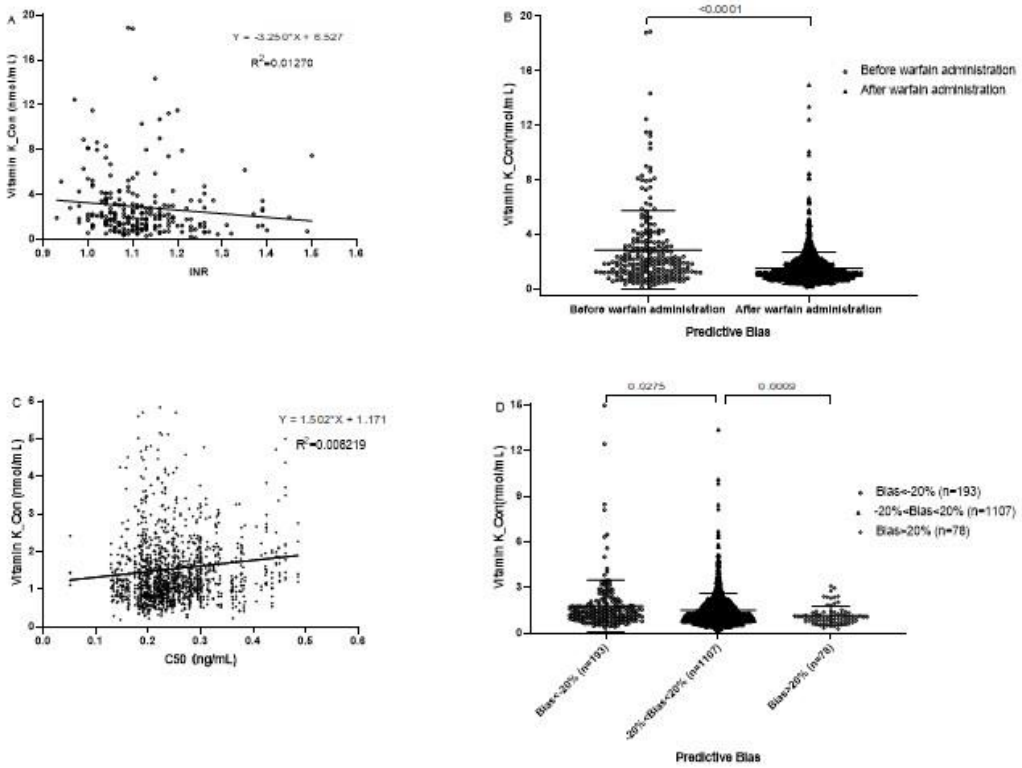

Figure 1. The relationship between the concentration of vitamin K and warfarin. A: VK concentration *versus* INR of prewarfarin administration; B: VK concentration *versus* prewarfarin and postwarfarin administration; C: VK concentration *versus* the pharmacodynamic parameter C50 of S-warfarin; D: VK concentration *versus* different INR PE.

Partial least squares discriminant Analysis (PLS-DA) was used to compare the differences in high prediction, ideal prediction and low prediction of INR PE at the OTU level. A total of 176 fecal samples were collected prewarfarin administration. A total of 446 fecal samples were collected postwarfarin administration. Only fecal samples of post-warfarin administration were analyzed for gut microbiota. There were 44, 359, and 43 fecal samples for high prediction, ideal prediction and low prediction, respectively. When analyzing the differences in gut microbiota in different groups, the sample size of each group should be balanced as far as possible, so the INR PE of the ideal prediction group was set as within ±5%. Finally, the number of ideal predictions for INR PE was 124. The results showed that the three groups had some differences (Figure 2).

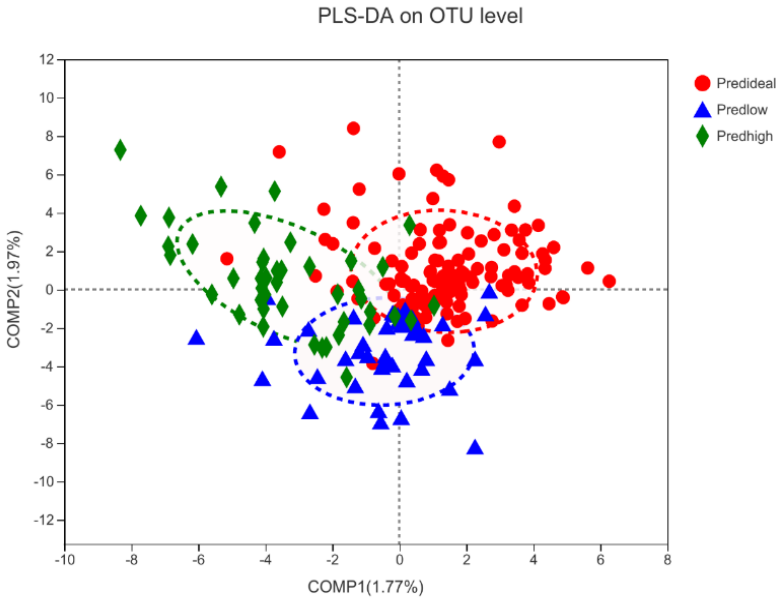

Figure 2. PLS-DA of gut microbiota for different INR PE

The top 20 most abundant bacterial species were compared for the difference of high prediction, ideal prediction and low prediction of INR PE at the genus level. The results showed that the bacterial species *Lactobacillus*, *Blautia*, *Faecalibacterium*, *Veillonella*, *Megamonas*, *Ruminococcus_gnavus_group*, *and Prevotella* showed significant statistic differences (P<0.05) (Figure 3). *Prevotella* is considered the source of vitamin K2 from gut microbiota (12).

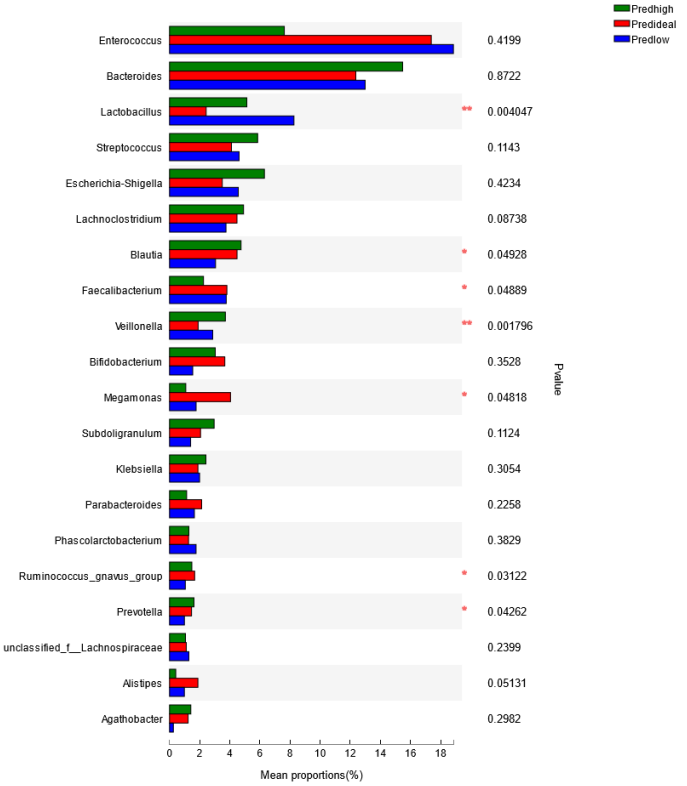

Figure 3. Comparison of the top 20 most abundant bacterial species for high prediction, ideal prediction and low prediction of INR PE.

Conclusions *(optional)*

Vitamin K had some effect on the individual variability of warfarin. The gut microbiota might also have a certain effect on the variability of warfarin.

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