

Clinical analysis of the effects of azole antifungal agents on the anticoagulant activity of warfarin

Wenjun Chen, BS^{a,b}, Tingting Wu, BS^{a,b}, Shaojun Jiang, BS^{a,b}, Meina Lv, BS^{a,b}, Jinglan Fu, BS^{a,b}, Xiaotong Xia, BS^{a,b}, Jinhua Zhang, PhD^{a,b,*}

Abstract

To investigate the frequency and degree of azole antifungal agents that influence the anticoagulant activity of warfarin to reduce the potential bleeding risk and provide a reference for rational administration of warfarin in clinics.

Patients with an abnormal international normalized ratio (INR; INR \geq 4.5) and treated with warfarin plus azole antifungal agents were screened from February 2011 to July 2016, and their data were extracted.

Thirty-two patients treated with warfarin plus azole antifungal agents were included. The INR of all the included patients increased by more than 20% of the INR of warfarin alone, and the warfarin sensitivity index showed an upward trend. The INRs of 21 patients treated with fluconazole (FLCZ) and warfarin was closely monitored for 1 week after the combination treatment, and the interaction between warfarin and the azole antifungal agents peaked on the seventh day. The INRs when warfarin was coadministered with azoles (Y) correlated significantly with those in the absence of azoles (X): FLCZ: $Y = 1.2515X + 2.1538$, $R^2 = 0.8128$; and voriconazole $Y = 2.4144 X + 2.6216$, $R^2 = 0.7828$.

The combination of FLCZ and voriconazole will enhance the anticoagulant effect of warfarin. Therefore, it is recommended to detect the genotype of CYP2C9 in patients and evaluate the interaction between the 2 drugs to adjust the warfarin dose. It is also recommended to closely monitor INR within 1 week of the addition of azole antifungal agents.

Abbreviations: FLCZ = fluconazole, INR = International normalized ratio, VRCZ = voriconazole, WSI = Warfarin sensitivity index.

Keywords: azole antifungal agents, drug-drug interaction, International normalized ratio, warfarin

1. Introduction

Warfarin has become the most commonly used oral anticoagulant for the prevention and treatment of thromboembolism, due to its remarkable and prolonged anticoagulant effect, ease of administration, and a low price. It is mainly used in the treatment of non-valvular atrial fibrillation or atrial flutter,^[1] pulmonary embolism, and deep vein thrombosis after artificial heart valve replacement. Its anticoagulant effect is activated by the inhibition of vitamin K-dependent coagulation factors.^[2] However, the

narrow treatment window of warfarin and the individualized differences among patients greatly affect the anticoagulant activity of warfarin in addition to many other factors, including genetic factors,^[3] dietary factors, drug interactions^[4] and complications, of which drug interactions have a greater impact.

Clinically, warfarin is often administrated with other drugs that have different effects on the anticoagulant activity of warfarin. When warfarin is combined with azole antifungal agents, the anticoagulant activity of warfarin enhances and the risk of bleeding increases, which may affect patients' lives in some cases. Only by fully understanding the frequency and extent of the interaction between azole antifungal drugs and warfarin and its effect on the anticoagulant activity of warfarin, can we predict the change of International normalized ratio (INR) after combined in advance and reduce the risk of bleeding. However, currently, there is less effective information on the frequency or degree of the interaction between azole antifungal agents and warfarin in clinical practice. Furthermore, preventive measures against the interaction between the drugs remain insufficient. In this study, we retrospectively analyzed the anticoagulant activity of warfarin after it was combined with different azole antifungal agents to provide a reference for a clinical evaluation of the frequency, degree, and time of such drug interactions and the warfarin dose adjustment method.

2. Data and methods

2.1. Ethics

This was a retrospective study, which was approved by the Ethics Committee of China Fujian Medical University Union Hospital (Fujian, China).

Editor: Nirmal Verma.

This study was supported by the Natural Science Foundation of Fujian Province of China [grant number 2018Y0037].

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

^a Department of Pharmacy, Fujian Medical University Union Hospital, Fuzhou, Fujian, ^b College of pharmacy, Fujian Medical University, Fuzhou, China.

* Correspondence: Jinhua Zhang, Department of Pharmacy, Fujian Medical University Union Hospital, #29 Xinquan Road, Fuzhou 350001, China (e-mail: pollyzhang2006@126.com).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Chen W, Wu T, Jiang S, Lv M, Fu J, Xia X, Zhang J. Clinical analysis of the effects of azole antifungal agents on the anticoagulant activity of warfarin. *Medicine* 2020;99:46(e22987).

Received: 21 June 2020 / Received in final form: 20 September 2020 /

Accepted: 1 October 2020

<http://dx.doi.org/10.1097/MD.00000000000022987>

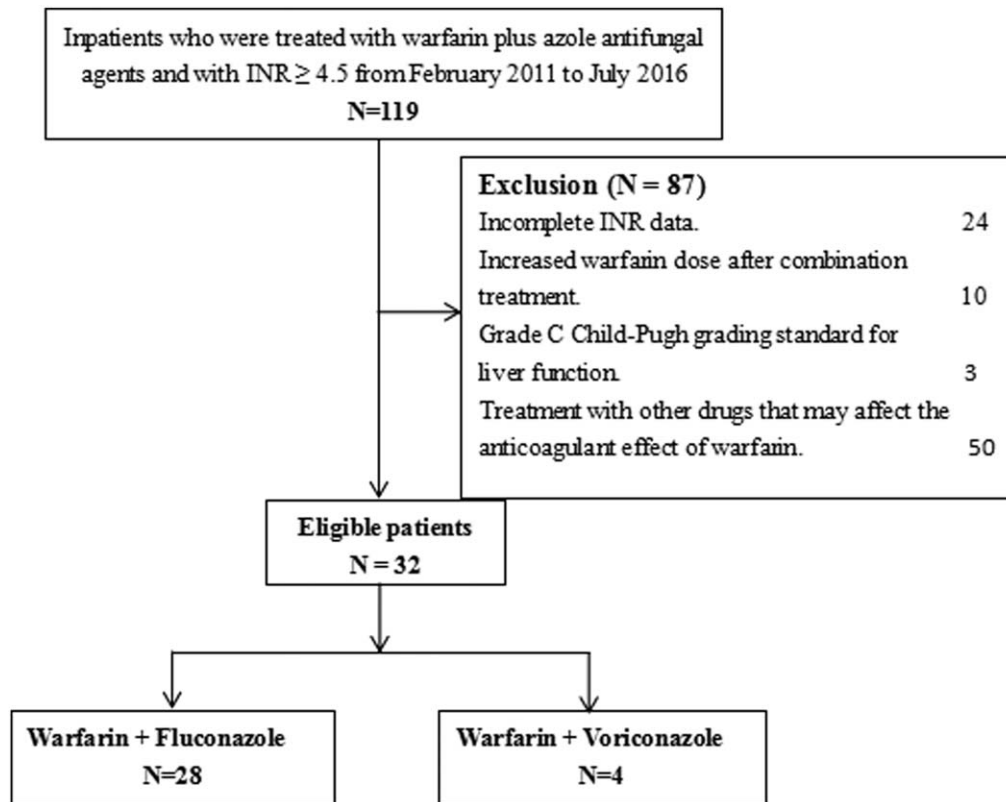


Figure 1. Screening process of the study population.

2.2. Data

Data of inpatients who had abnormal INR ($\text{INR} \geq 4.5$) and were treated with warfarin plus azole antifungal agents from February 2011 to July 2016 in a teaching hospital in China were extracted.

2.3. Methods

2.3.1. Screening of the study population. Patients were recruited according to inclusion criteria. The screening process is shown in Figure 1.

Inclusion criteria: ① Patients for whose complete INR record was available; ② Patients with grade A or grade B Child-Pugh grading standard for liver function; ③ Patients for whom warfarin dose did not increase after combination with azole antifungal agents; and ④ patients who received only azole antifungal agents plus warfarin.

2.3.2. Information collection. Sex, age, doses of warfarin and azole antifungal agents, reasons for using warfarin and azole antifungal agents, liver function index value during combination therapy, INR before and after combination therapy, and combination therapy used were collected from patients who meet inclusion criteria.

INR of warfarin alone was defined as INR with a stable warfarin dose within 2 weeks before warfarin combination with azole antifungal agents or other drugs that may affect the anticoagulant effect of warfarin, or INR with a stable warfarin dose alone at 2 weeks after the end of the combination treatment. INR of the combination was defined as the maximum INR during the warfarin plus azole antifungal agents. If the emergency

measure of injecting vitamin K1 was taken during the combination treatment, the highest INR before the injection of vitamin K1 was recorded. Since the warfarin dose was adjusted for some patients, we evaluated the effects of azole antifungal agents using the Warfarin sensitivity index (WSI),^[5] calculated as $\text{INR value/daily warfarin dose 1 day prior to the measurement of INR}$. If the INRs of the patients increased by $>20\%$, azole antifungal agents were considered to have enhanced the effect of warfarin.^[6]

2.4. Statistical analysis

Changes in INR and WSI were analyzed using paired t-test by SPSS23.0. Values of $P < .05$ were considered statistically significant.

3. Results

3.1. Study population

The clinical characteristics of the included subjects were extracted according to inclusion criteria as shown in Table 1.

3.2. Changes of INR or WSI before and after fluconazole (FLCZ) or voriconazole (VRCZ) were combined

The changes in INR or WSI before and after combination with FLCZ in 28 patients and VRCZ in 4 patients are shown in Figure 2. The INR of all the included patients increased by more than 20% of the INR of warfarin alone. The mean duration of

Table 1
Study subject characteristics.

Azole antifungal agent	FLCZ	VRCZ
Total cases (male)	28 (11)	4 (3)
Age (yr)	60 (33–88)	73 (70–79)
Azole dose (mg/d)	200 (50–400)	195 (150–200)
Warfarin dose (mg/d):		
Warfarin alone	2.5 (0.875–4.5)	2.375 (1.5–3.0)
With azole antifungals	2.25 (0.875–3.75)	2.285 (0.75–5.25)
Reason for warfarin administration		
Deep vein thrombosis	2	0
Atrial fibrillation	3	3
Heart valvular regurgitation	3	0
Post-valve-replacement	2	0
Cardiogenic cerebral infarction	3	1
Rheumatic cardiopathy	15	0
Reason for azole administration		
Candida albicans fungus	28	0
Aspergillus infection	0	4

FLCZ = fluconazole, VRCZ = voriconazole.

warfarin in combination with FLCZ was 10 days (2–29 days). The mean INR of the patients increased by 2.5-fold ($P < .001$) from 1.7 to 4.3. Further, the WSI value increased from 0.79 to 2.14, an increase of 2.7-fold ($P < .001$). The mean duration of

warfarin and VRCZ combination treatment was 11 days (range: 3–18 days). The mean INR of the patients increased from 1.27 to 5.7, an increase of 4.3-fold higher ($P < .005$). The average WSI of the patients increased from 0.6 to 2.5, an increase of 4.1-fold ($P < .05$).

3.3. Effects of azole agents on the anticoagulant activity of warfarin

Among the 28 patients treated with FLCZ and warfarin, INR of all patients increased by more than 20% on day 7 after the combination treatment. INR values of 21 patients receiving warfarin plus FLCZ were closely monitored in the first week. All patients underwent INR measurements on the third day after the combination treatment, 8 patients were tested on the fifth day, and 5 patients were tested on the seventh day. As shown in Figure 3, the INR of all patients showed an upward trend. The INR of 18 patients exceeded 100% of that observed with warfarin alone on day 3 after co-administration. The INR of 5 patients exceeded 200% of the INR of warfarin alone on day 5, and the interaction between warfarin and FLCZ peaked at 400% of the INR of warfarin alone.

Four patients who received VRCZ were included, whose INR was not closely tested within one week, and the data were insufficient to analyze the degree of influence.

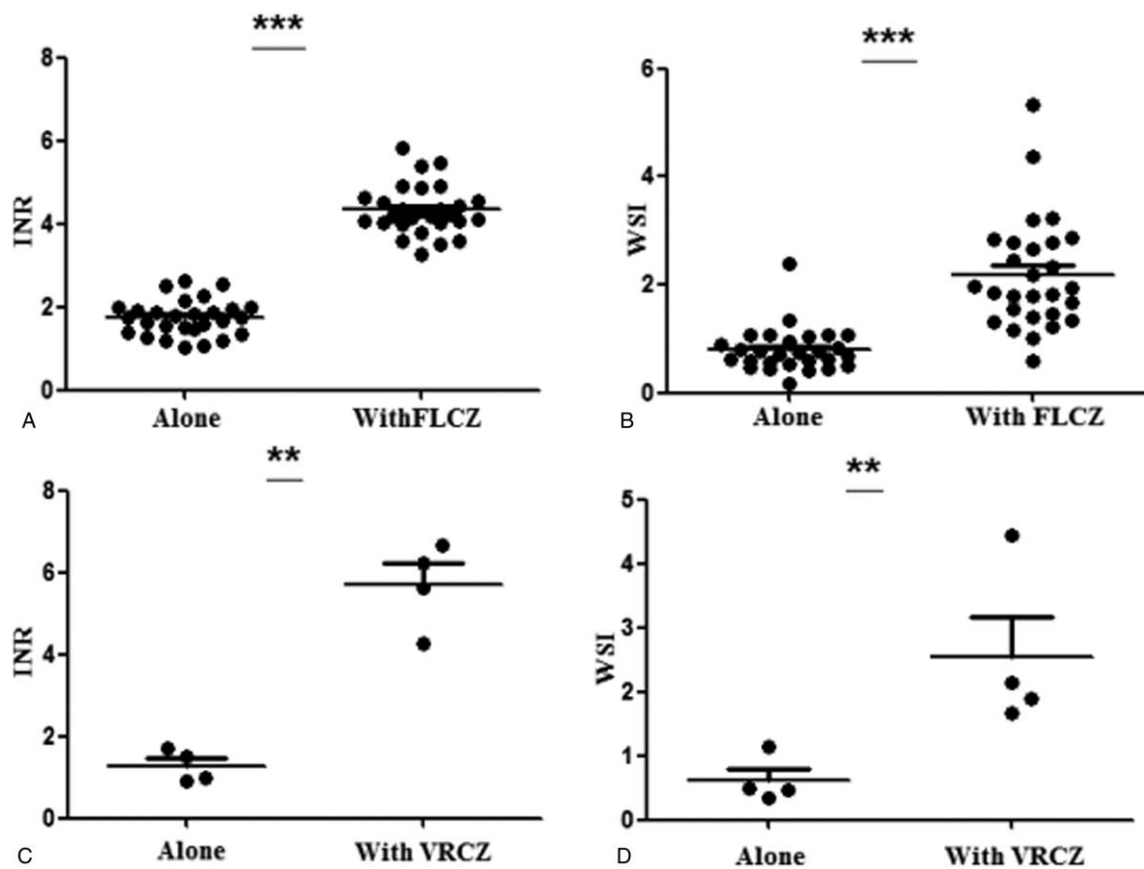


Figure 2. Comparison of INR and WSI in patients using warfarin alone and with FLCZ and VRCZ. A and B show the comparison of INR and WSI of warfarin alone and upon combination with FLCZ, respectively. INR and WSI of warfarin alone and for combination with VRCZ are compared in C and D, respectively. Each horizontal bar shows the mean value. $P < .05$, $***P < .001$.

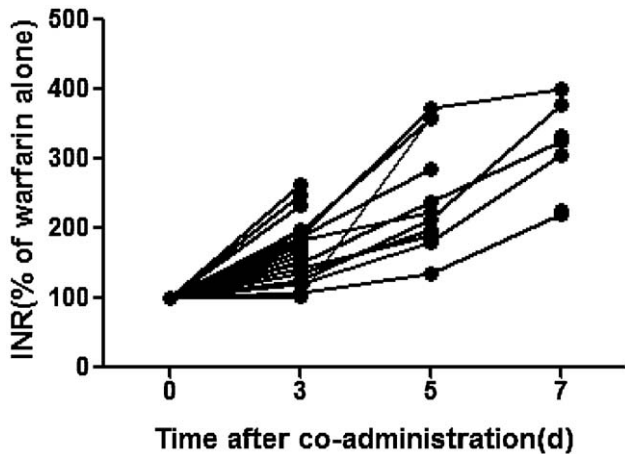


Figure 3. Changes in INR of 21 patients after co-administration of FLCZ. INR was expressed as the percentage before the combined use of FLCZ.

3.4. Relationship of INR or WSI between warfarin monotherapy and co-administration of FLCZ or VRCZ

Linear analysis was performed with the INR or WSI of patients using warfarin alone as the X value and the INR or WSI of patients using warfarin plus FLCZ or VRCZ as the Y value, and the results are shown in Figure 4. The linear relationships for INR are shown in Figure 4 (A) as follows: FLCZ, $Y = 1.2515X + 2.1538$, $R^2 = 0.8128$; VRCZ, $Y = 2.4144x + 2.6216$, $R^2 = 0.7828$. The corresponding relationships for WSI are shown in Figure 4 (B) as follows: FLCZ: $Y = 1.9347x + 0.5996$, $R^2 = 0.5599$, VRCZ: $Y = 3.4663x + 0.4334$, $R^2 = 0.929$.

4. Discussion

In this study, we analyzed the INR and WSI values of patients before and after combined use of warfarin and azole antifungal agents. The INR and WSI of patients significantly increased after addition of FLCZ, which enhanced the anticoagulant effect of warfarin. This is consistent with the findings reported by

Yamamoto et al^[6] who observed that patients treated with warfarin had an increased risk of bleeding following FLCZ administration. The study conducted by Yamamoto et al.^[6] in the Japanese population found that the addition of VRCZ could increase the WSI of hospitalized patients, but there was no statistically significant difference. In this study, INR and WSI of the patients significantly increased after the addition of VRCZ, and the increase was even stronger than that observed with FLCZ, with statistical significance. In the 21 cases that were selected according to the inclusion criterion, the INR and WSI of patients showed an upward trend as early as day 1 after the combined application of FLCZ or VRCZ and generally reached a peak 1 week after the combined application. As shown in Figure 3, the INR in half of the patients receiving FLCZ had increased to 150% of that recorded with warfarin alone, and the INR in 1/7th of the patients increased to 200% on day 3, while that in 1/4th of the patients increased to 200% on day 5. These changes in INR significantly increased the risk of bleeding in patients.

Studies have shown that the inhibitory effect of azole antifungal drugs on liver drug metabolism enzymes is the main mechanism by which azole antifungal drugs enhance the anticoagulant activity of warfarin, leading to an increased risk of bleeding in patients.^[7] Warfarin is a racemic mixture with R- and S-isomers. R-warfarin is mainly metabolized by CYP1A2 and CYP3A4, and 85% of S-warfarin is metabolized by CYP2C9. As a vitamin K antagonist, the activity of S-isomers warfarin is more than 5 times higher than that of R-isomers warfarin,^[8] so CYP2C9 activity has a substantial influence on the anticoagulant effect of warfarin. FLCZ and VRCZ act on the hepatic cytochrome P450 system. The anticoagulant effect of warfarin was enhanced by inhibition of CYP2C9 to reduce the degradation of warfarin.

In in vitro experiments, the IC_{50} values of FLCZ and VRCZ for inhibition of CYP2C9 were 9.3 mg·L⁻¹ and 8.4 mg·L⁻¹,^[9] respectively. When FLCZ was administered at a dose of 200 mg daily, the blood concentration of FLCZ was 2.8~6.9 mg·L⁻¹,^[10] and when 400 mg VRCZ was administered daily, the blood concentration of VRCZ was 1~6 mg·L⁻¹.^[11] Although IC_{50} is equivalent to or slightly higher than the plasma concentration of treatment, FLCZ and VRCZ are highly likely to interact with

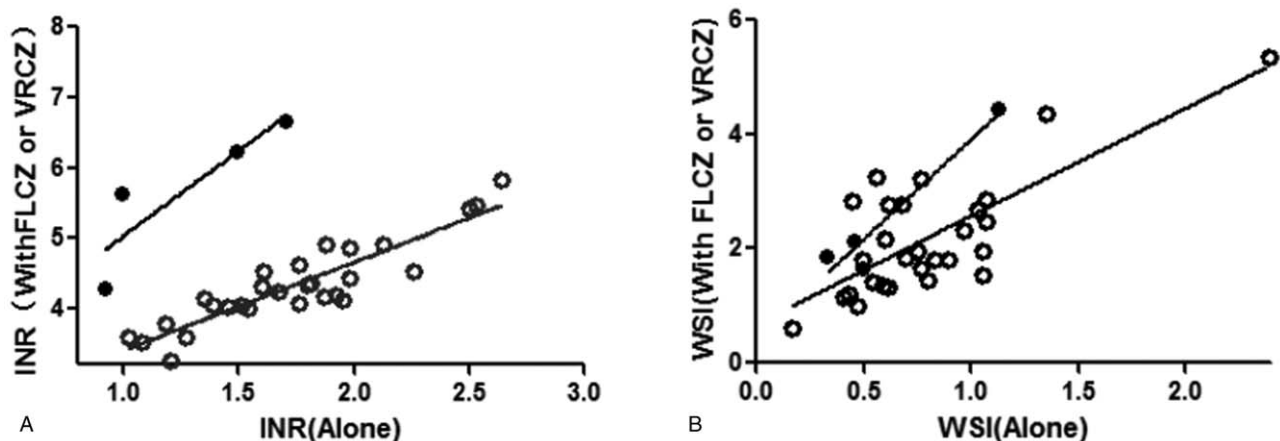


Figure 4. Relationship of INR or WSI between warfarin monotherapy and co-administration of FLCZ or VRCZ. Open and closed circles represent the values in patients administered FLCZ and VRCZ. Linear regression line determined by the least squares method. INR is shown in (A): FLCZ: $Y = 1.2515X + 2.1538$, $R^2 = 0.8128$; VRCZ: $Y = 2.4144x + 2.6216$, $R^2 = 0.7828$. WSI is shown in (B): FLCZ: $Y = 1.9347x + 0.5996$, $R^2 = 0.5599$. VRCZ: $Y = 3.4663x + 0.4334$, $R^2 = 0.929$.

drugs metabolized by CYP2C9. Currently, the Food And Drug Administration (FDA) considers both FLCZ and VRCZ as inhibitors of CYP2C9. The warfarin instructions mention that the combination of warfarin and azole antifungal agents can enhance the anticoagulation effect of warfarin, thus increasing the risk of bleeding. Therefore, when warfarin is combined with FLCZ or VRCZ, changes in INR value should be closely monitored to reduce the risk of bleeding.

Itraconazole, ketoconazole and miconazole are also the commonly used azole antifungal agent in clinical practice. In this study, no case of warfarin combined with those drugs were retrieved, so the interaction between the those drugs and warfarin could not be studied. It is generally believed that itraconazole is a strong suppressant of CYP3A4, with limited inhibitory effect on CYP2C9.^[10] Therefore, itraconazole can affect the metabolism of R-isomers warfarin, but since S-isomers warfarin shows 5 times greater anticoagulant activity than R-isomers warfarin, the effect of itraconazole on the anticoagulant activity of warfarin is theoretically limited. The study by Yamamoto et al.^[6] also suggested that itraconazole had no significant effect on the anticoagulant activity of warfarin. However, itraconazole has been reported to increase the blood concentration of S-warfarin.^[12] Besides, ketoconazole is a strong suppressant of CYP3A4, with limited inhibitory effect on CYP2C9. In JACKEVICIUS CA's study, ketoconazole was shown to increase the anticoagulant activity of warfarin.^[13] Miconazole also has a significant effect on warfarin for its inhibition on CYP2C9.^[14] Therefore, in order to avoid clinical adverse events, INR values should also be closely monitored in patients receiving the combination of warfarin and azole antifungal agents.

Studies have suggested that the cytochrome *P450 2C9* genotypes are associated with the dose requirements of warfarin, and risk of bleeding.^[15] Meanwhile, this may lead to different effects of the interaction between azole antifungal agents and warfarin. The genotypes of *CYP2C9* include *CYP2C9**1/*1, *1/*3, *3/*3. They belong to 3 metabolic phenotypes: rapid metabolism type(*1/*1), intermediate metabolism type(*1/*3), and slow metabolism type(*3/*3). Adjusting the dose of warfarin according to the patient's genotype can significantly reduce the risk of bleeding.^[15] According to the pharmacokinetic theory, it is speculated that patients with *CYP2C9* genotype of slow metabolism type(*CYP2C9**3/*3), the interaction between warfarin and azole antifungal drugs will be weakened; for patients with intermediate metabolism type(*1/*3), interaction generally; for patients with rapid metabolism type(*1/*1), interaction will be promoted.^[16] Therefore, clinically, the effect of azole antifungal drugs on the anticoagulant effect of warfarin can be evaluated by detecting the genotype of *CYP2C9* in patients. And the warfarin dose can be adjusted based on the test results to reduce the risk of bleeding. For patients with rapid metabolism type(*1/*1), the dose of warfarin should be reduced when warfarin and azole antifungal drugs are combined.

The linear relationship between INR and WSI before and after the addition of FLCZ and VRCZ was also analyzed. The linear relationship between INR and WSI before and after the addition of FLCZ in 28 patients and VRCZ in 4 patients was analyzed. The linear indexes of INR were as follows: FLCZ: $R^2 = 0.8128$; VRCZ: $R^2 = 0.7828$. The linear indexes of WSI were as follows: FLCZ: $R^2 = 0.5599$; VRCZ: $R^2 = 0.929$. This indicated the presence of a linear relationship between INR and WSI before and after warfarin was combined with azole antifungal agents, which was consistent with the results reported by Yamamoto

et al.^[6] Patients with higher INR while receiving warfarin monotherapy are at greater risk of bleeding after the addition of azole antifungal agents, and should be closely monitored. However, due to the small sample size, the linear relation obtained in this study is not optimal. Thus, a stable linear relationship was obtained through continuous data collection to explore a new warfarin dose adjustment method, to maintain the INR value within the normal range and reduce the risk of bleeding.

5. Conclusion

In conclusion, when warfarin was combined with FLCZ or VRCZ, the anticoagulant activity of warfarin was significantly enhanced and reached its peak after 1 week of combined use. Therefore, it is recommended to detect the genotype of *CYP2C9* in patients and evaluate the interaction between the 2 drugs to adjust the warfarin dose. Meanwhile, it is also recommended to closely monitor INR within 1 week of the addition of azole antifungal agents.

Author contributions

All authors contributed to the study conception and design. Wenjun Chen, Tingting Wu and Shaojun Jiang designed this study. Material preparation and data collection were performed by Meina Lv, Jinglan Fu and Xiaotong Xia. The first draft of the manuscript was written by Wenjun Chen and all authors commented on previous versions of the manuscript. Jinhua Zhang revised the manuscript. All authors read and approved the final manuscript.

Conceptualization: Wenjun Chen, Jinhua Zhang.

Data curation: Tingting Wu, Shaojun Jiang, Meina Lv, Jinglan Fu, Xiaotong Xia, Jinhua Zhang.

Software: Tingting Wu.

Validation: Tingting Wu.

Writing – original draft: Wenjun Chen.

References

- Clark NP, Hoang K, Delate T, et al. Warfarin interaction with hepatic cytochrome p-450 enzyme-inducing anticonvulsants. *Clinical and Applied Thrombosis/Hemostasis* 2017;107602961668784.
- Hirsh J, Dalen JE, Deykin D, et al. Oral anticoagulants. Mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest* 1995;108(4 Suppl):231S.
- Gage B, Eby C, Johnson J, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin[J]. *Clinical Pharmacology & Therapeutics* 2008;84:326–31.
- Holbrook , Anne M. Systematic overview of warfarin and its drug and food interactions. *Archives of Internal Medicine* 2005;165:1095.
- Halkin H, Shapiro J, Kurnik D, et al. Increased warfarin doses and decreased international normalized ratio response after nationwide generic switching. *Clinical Pharmacology & Therapeutics* 2003;74:215.
- Yamamoto H, Habu Y, Yano I, et al. Comparison of the effects of azole agents on the anticoagulant activity of warfarin. *Biological and Pharmaceutical Bulletin* 2014;37:1990–3.
- Niwa T, Shiraga T, Takagi A. Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes. *Biological & Pharmaceutical Bulletin* 2005;28:1805–8.
- Ansell J, Hirsh J, Hylek E, et al. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Ed.). *Chest* 2008;133:160S–98S.
- Niwa T, Shiraga T, Takagi A. Effect of drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes. *Biological & Pharmaceutical Bulletin* 2005;28:1805–8.

- [10] Vand EKCM, Span LFR, Van Hateren K, et al. Dried blood spot analysis suitable for therapeutic drug monitoring of VRCZ, FLCZ, and posaconazole. *Antimicrobial Agents and Chemotherapy* 2013;57:4999–5004.
- [11] Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrobial Agents and Chemotherapy* 2009;53:24–34.
- [12] Miura M, Takahashi N, Kanno SI, et al. Drug interaction of (S)-warfarin, and not (R)-warfarin, with itraconazole in a hematopoietic stem cell transplant recipient[J]. *Clinica Chimica Acta* 2011;412:0–2006.
- [13] Jackevicius CA, Ton MN. Enhanced interaction between warfarin and high-dose ketoconazole: a case report [J]. *Case Reports Med* 2014;2009:315687.
- [14] Tajimaokubor , Tsuruoka S, Moriyama N, et al. Interaction of miconazole oral gel with warfarin and cyclosporine in a patient with nephrotic syndrome. *Cen Case Rep* 2012;1:55–7.
- [15] Jinhua Zhang, Tingting Wu, Wenjun Chen, et al. Effect of gene-based warfarin dosing on anticoagulation control and clinical events in a real-world setting. *Pharmacol* 2020.
- [16] Guo RT, Yu F, Xu H, et al. Research progress on the effect of proton pump inhibitors on warfarin anticoagulant effect. *Pharm Clin Stud* 2015;23:479–83.