Laboratory monitoring of warfarin in the era of direct oral anticoagulants

Originally developed after cattle that were fed a sweet clover derivative had fatal bleeding and subsequently introduced more than 60 years ago as a pesticide against rats and mice, warfarin gained wide popularity as an anticoagulant because of its effective inhibition of blood coagulation, low cost, and broad availability.[1] In the emerging era of therapeutic management of patients with either venous thromboembolism or atrial fibrillation with direct oral anticoagulants and innovative drugs designed to specifically target and inhibit activated coagulation factors II and X, warfarin remains a mainstay of anticoagulant therapy, especially in low-income countries and in many select patients including those with impairment of kidney and liver function.[2] The anticoagulant effect of warfarin can also be efficaciously reversed by administration of vitamin K, whereas no effective antidotes are available for direct oral anticoagulants. However, warfarin presents a number of drawbacks compared with direct oral anticoagulants, including delayed onset of anticoagulant effect, longer half-life and, especially, the narrow therapeutic range, which needs constant laboratory monitoring.[2]

The anticoagulant activity of warfarin is mostly attributable to inhibition of vitamin K, an essential cofactor in the process of γ-carboxylation of clotting factors II, VII, IX, and X, a biochemical modification that is essential for these factors to bind to negatively charged surfaces (eg, platelets or synthetic phospholipids), by which their activation can be amplified and thus more efficiently triggered.[3] Because factor VII has the shortest half-life (about 3–6 h), its inactivation is the most limiting step in the in-vitro anticoagulant effect of warfarin. Therefore, unsurprisingly, the prothrombin time (PT), with its further modification by means of international normalised ratio (INR, a mathematical calculation to attenuate inter-laboratory and inter-instrumental variability), has represented the gold standard for routine monitoring of warfarin for decades. The leading drawback in this approach is that although a prolongation of the INR reflects the fluctuation of all vitamin K-dependent factors, the fluctuation of factor VII is prevalent because of its short half-life, whereas the anticoagulant effect of warfarin in vivo is instead prevalently attributable to factors II, X, and IX.[4] The high sensitivity of the INR to factor VII is a major drawback that increases INR fluctuation and reduces patient time within the therapeutic range.

In *The Lancet Haematology*, Onundarson and colleagues[5] report the development of an innovative test for monitoring the anticoagulant effect of warfarin, called Fiix-prothrombin time (Fiix-PT), which is essentially based on the measurement of factor II and X activity, thus almost insensitive to inactivation of factor VII in plasma.[5] They identified that during day 1–720, ten (1·2% per patient-year) thromboembolism events occurred in the Fiix-PT group versus 19 (2·3% per patient-year) in the PT group (relative risk [RR] 0·52; 95% CI 0·25–1·13, p<0·0001 for non-inferiority). The results are very encouraging because this novel test was proven to be at least as effective as the INR for monitoring of warfarin, while also producing improved dosing stability and a decreased risk of thromboembolism with no increased risk of bleeding. At variance with other more expensive and labour intensive techniques, such as thrombin generation, the new method was developed with conventional laboratory instrumentation, which would make it accessible to most clinical laboratories and, hypothetically, to point of care devices as well.

Importantly, the Fiix-PT exhibited overall non-inferior efficacy for primary outcomes, meaning it did as well as the INR in clinical terms. Patient monitoring was only reduced by 5·8% and, although reaching statistical significance, the interval between tests was reduced by 1 day, whereas the percentage of tests within target range was reduced by 2·0% and the frequency of tests with results lower than the optimum therapeutic range was reduced by 2·9%. Conversely, Fiix-PT also improved anticoagulation and dosing stability, and post-hoc analysis suggested that long-term thromboembolism was reduced without increasing bleeding.

To translate these results into practical patient management, a cost-effectiveness analysis would be necessary to define whether these improvements reflect sufficient incremental benefit compared with

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the alternative of direct oral anticoagulants, which do not require laboratory monitoring. At variance with reagents used for the INR, on the basis of thromboplastin, a source of phospholipids and calcium,[6] the Fiix-PT also needs mixing of the patient sample with factor II and X double-depleted plasma, increasing test cost. Also, the calibrator used for Fiix-PT was designed for standardisation of PT, and potential deviance from test methods might need a specific set of calibrators and controls for the novel test. Demonstration of accuracy and reproducibility of data between different laboratories is also needed before the clinical use of Fiix-PT can be more widely validated and used. This problem was clearly shown in the study[5] because a calibration problem in the international sensitivity index was not detected in a timely manner with conventional internal quality control.

Therefore, although the encouraging results published by Onundarson and colleagues[5] certainly deliver potential for reinvigorating clinical use of warfarin, more research is needed to establish whether the Fiix-PT is ready for widespread use.

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We declare no competing interests.

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