Warfarin’s monopoly on oral anticoagulation therapy, as well as that of other vitamin K antagonists derived from coumarin, is over. Two new classes of drugs, oral direct thrombin inhibitors (DTI)\(^1\) and oral direct factor Xa inhibitors (DFXaI), are steadily obtaining regulatory approval for prevention of thromboembolic events in patients who have atrial fibrillation, undergo hip or knee replacement surgery, or require treatment of pulmonary emboli and deep vein thromboses. The results from an expanding list of large, well-conducted randomized clinical trials confirm the efficacy and safety of DTI and DFXaI drugs as noninferior or superior to standard prophylactic or therapeutic anticoagulation therapies \((1)\). Presently, the US Food and Drug Administration (FDA) and regulatory agencies in other countries have approved one DTI (dabigatran etexilate, Pradaxa\(^6\)) and two DFXaI (rivaroxaban, Xarelto\(^8\); apixaban, Eliquis\(^8\)) for one or more indications, and clinical trials that were recently completed or are underway will likely bring more drugs in these two classes to market with approval for additional indications \((2)\).

The common mechanism of action of these small molecules is reversible blockage of the active enzyme site of thrombin or factor Xa. Thrombin converts fibrinogen to fibrin, and factor Xa converts the zymogen prothrombin to thrombin. Inhibition of either coagulation factor reduces the rate of fibrin clot formation. Unlike warfarin or heparin, DTI and DFXaI activities are not dependent on decreased synthesis of coagulation factors or acceleration of the effects of antithrombin, an endogenous inhibitor of thrombin and factor Xa.

DTI and DFXaI have many advantages compared with coumarins, including rapid onset of anticoagulation, no dietary restrictions, few drug–drug interactions, and more predictable pharmacokinetics. As a result, they are prescribed as fixed doses without adjustments on the basis of laboratory monitoring, and there are no therapeutic ranges to target.

But no drug is perfect, and DTI and DFXaI have some disadvantages: partial renal excretion requiring dose reduction or complete exclusion of the drug from the therapeutic regimen depending on the severity of kidney dysfunction; the lack of a rapid reversal agent or antidote; and the unavailability of FDA-approved companion diagnostic tests to measure their concentration or in vitro anticoagulant activity in specific situations such as suspected overdose or noncompliance, life-threatening bleeding, or before invasive procedures. In addition, because drug monitoring was not performed in clinical trials, concentration thresholds for increased risks of bleeding or thrombosis are not known.

Another consequence of DTI and DFXaI therapy is interference with screening coagulation tests performed on undiluted plasma and with some specialized clot-based and chromogenic coagulation tests. In this issue of *Clinical Chemistry*, Helin and colleagues report their findings when normal pooled plasmas, spiked with dabigatran (the active metabolite of dabigatran etexilate, a DTI), or rivaroxaban (a DFXaI) were sent to laboratories participating in a European external quality assessment program \((3)\). Their analysis focused on prothrombin time (PT) and activated partial thromboplastin time (APTT) results. Participants used 13 different thromboplastins to perform PTs and 9 different APTT reagents when testing the dabigatran-spiked samples and 5 PT and 4 APTT reagents when testing the rivaroxaban-spiked samples. The PT was less sensitive than the APTT to both anticoagulants, and there was considerable reagent-dependent variability in response to higher drug concentrations (relative increase over upper limit of local reference interval) for both tests. Thromboplastins based on the Owren method, primarily used in Scandinavian countries, were particularly insensitive to dabigatran. APTT reagents were sensitive to plasma containing 120 µg/L dabigatran, with 97% of APTTs above the local reference interval. APTT reagents were modestly sensitive to plasma containing 60, 146, and 305 µg/L rivaroxaban. The APTT prolongations were curvilinear with both anticoagulants. These findings are generally consistent with results from other in vitro spiking studies, with a few exceptions \((4–8)\).
Quantitative methods to measure DTI and DFXaI anticoagulant activities are expensive and require local validation. Therefore, is it reasonable to use their interference with PT or APTT results to derive clinically useful conclusions regarding bleeding or thrombosis risk? The data of Helin et al. (3) suggest that the PT is not very useful for identifying low or high concentration extremes of DTI and DFXaI owing to poor sensitivity and variable responsiveness. Other studies confirm that APTT is insensitive to high concentrations of dabigatran and rivaroxaban (>150–200 µg/L) owing to its curvilinear response (5, 8). The data of Helin et al. (3) might suggest that if the APTT is not prolonged, it could be sensitive enough to rule out a clinically meaningful concentration of dabigatran. However, the lower spiked dabigatran concentration (120 µg/L) is within the wide peak range (64–443 µg/L) reported during clinical trials (9), and although there is no consensus regarding a concentration of dabigatran (or rivaroxaban) that poses a negligible bleeding risk, it is likely to be <120 µg/L. In addition, Helin et al. (3) performed APTTs on plasmas from 10 patients taking dabigatran (mean concentration 119 µg/L, range 100–135.5 µg/L). The APTT results were significantly lower than those (with the same commercial reagent) from plasma spiked with 120 µg/L, including 1 APTT within the reference interval, highlighting the challenges of using a global clotting test that can be affected by multiple variables to estimate a drug’s anticoagulant activity. For example, increased factor VIII activity can shorten, and factor depletion can prolong, APTT results. Helin et al. (3) obtained similar results when APTTs were performed on plasmas from 10 patients taking rivaroxaban (mean 63 µg/L, range 32–128 µg/L): all APTTs were within the reference interval. Therefore, one should not assume that a “normal” APTT excludes a DTI or DFXaI concentration high enough to pose an increased bleeding risk.

So, what are the responsibilities of a laboratory director supervising coagulation tests in the postwarfarin, unmonitored oral anticoagulation therapy era?

- Know the sensitivity (concentrations of DTI and DFXaI producing PTs and APTTs above the reference interval) and responsiveness (DTI and DFXaI concentrations to double PT and APTT from baseline) of your laboratory’s reagent/instrument combinations. This can be done by purchasing calibrators or enrolling in proficiency testing programs. Ex vivo spiked data are artificial, but they do provide some guidance.
- Share this information with clinicians, especially those most likely to encounter injured, bleeding, or preoperative patients (emergency medicine, trauma surgery, neurosurgery, anesthesiology), and help them interpret patient results in clinical context, including renal function and interval between last dose and blood collection, to estimate drug clearance rate.
- Rely on a “normal” thrombin time to rule out clinically relevant concentrations of dabigatran, because this test is sensitive to very low concentrations (approximately 25 µg/L) (4). Likewise, direct factor Xa inhibitors will cause interference in a chromogenic anti-Xa assay calibrated to monitor heparins, making this fairly common test suitable for screening (10).
- Evaluate the local utility and practicality of validating currently available methods to quantify the anticoagulant activity of DTI (dilute thrombin time or ecarin time calibrated with dabigatran) and DFXaI (chromogenic anti-Xa assay calibrated with rivaroxaban or apixaban). Appropriate clinical situations for such specialized, calibrated assays are likely to be uncommon and require urgent management decisions, i.e., stat performance, which is a challenging combination for most hospital laboratories.
- Through ex vivo testing and literature review (11), be aware of how DTI and DFXaI can interfere with other coagulation tests performed in the laboratory. Develop procedures to avoid these “contaminants” and provide appropriate caveats with test interpretations.
- Finally, stay current with clinical and laboratory progress in oral anticoagulation therapy. As use of DTI and DFXaI increases, management guidelines will evolve, pharmaceutical antidotes may arrive, and new quantitative diagnostic tests will appear that may be more applicable to routine clinical laboratories.

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References


