EDUCATIONAL COMMENTARY – RAT POISON, GENETICS, AND MOLECULAR BIOLOGY: WHITHER THE FUTURE OF COAGULATION TESTING?

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- understand why the effect of warfarin is so variable and why INR results vary so much.
- discuss the new genetic (molecular) tests that attempt to predict some of this variation and lead to shorter times to attain therapeutic INR levels.
- identify three new drugs that are warfarin ‘replacements’ and claim to require no routine monitoring.
- understand the effects that the new agents may have on the PT and aPTT.

Coagulation, being the absolutely miraculous system that it is, is far more complicated than depicted in textbook diagrams. The system includes lively endothelium; fuzzy, active, living platelets; flowing blood; and many other factors in addition to our favorite coagulation factors (numbers I-XIII). The coagulation system plugs holes, scrapes, and rips in various parts of our bodies with a nice neat blood clot, and then, just as importantly, stops the clotting. Without the checks and balances to stop and then clear (lyse) the clots, our entire vascular system would clot up the first time we scratched our finger! Because the coagulation system is so very complex and delicately balanced, we run the risk of tilting the balance too far towards either bleeding or clotting any time we try to modify it.

Case Study One

An acting coroner received a courtesy call from the emergency room physician informing him that an 82-year-old woman, on warfarin anticoagulation therapy, was eating lunch with her husband when she fell over and passed out. She died shortly thereafter. CT scan showed a brain stem hemorrhage. Her INR was 6.5 (therapeutic range 2-3). The physician wondered, “Why could her INR have been outside the therapeutic range? Did her high INR contribute to her death?”

Warfarin: The Yin and the Yang

What is warfarin? It is a white crystalline compound, introduced in 1948 as a rat poison, then six years later introduced as the first practical oral anticoagulant. All previous anticoagulants had to be injected. It was named after the W(isconsin) A(lumni) R(esearch) F(oundation) + (coum)arin.
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The “yang” of warfarin – warfarin was, and still is, a miracle drug.
- It has prevented strokes in millions of people.
- It has allowed artificial valves and even artificial hearts to be used, allowing practical treatment of additional millions of patients suffering from damaged heart valves or failing hearts.
- It has been used to treat millions of patients with pulmonary emboli (blood clots in the lungs) and deep venous thromboses (clots in the legs).
- It has saved hundreds of thousands to millions of lives.

The “yin” of warfarin – warfarin is also
- one of our most dangerous drugs.
- a drug that has led to thousands of deaths.
- a drug that frequently leads to bleeding complications.
- the most common cause of emergency hospitalizations for adverse drug reactions in the USA.

Warfarin: Disadvantages

The biggest disadvantage of warfarin is the wide variation in dosage from person to person. This is due to both genetics and interactions with foods, medicines, and herbal supplements. As a result, it may take months to establish the correct dose of warfarin for a patient. In the meantime the patient is at increased risk for clotting and bleeding. Overall, about one in five patients ends up with a major or minor bleeding event while on warfarin.

Genetic Variation

We each inherit genes that code for the metabolism or ‘disposal’ of warfarin. These genes account for approximately 15% of the total dose variation. First, warfarin is broken down in the liver by what is called the “CYP2C9” (CYtochrome P450 2C9) gene. There are more than 50 slightly different variants of the CYP2C9 gene. Some break down warfarin very quickly, while others are slow to get warfarin out of the system. A second and even bigger inherited contributor to warfarin dosing is variation in the target molecule. The target molecule is called “Vitamin K epoxide reductase complex subunit 1” or “VKORC1.” There are at least three clinically important variants of VKORC1, each of which is variably sensitive to warfarin. Together, CYP2C9 and VKORC1 account for about a third to half of the variation in warfarin dosing. The commonly available molecular genetic test “Warfarin sensitivity testing panel” tests for the clinically important variants of both of these important molecules.

Unfortunately, despite initial enthusiasm and a nudge from the FDA, genetic testing for warfarin dosing has not caught on very much in the USA. Medical studies have shown that you can get a patient into the therapeutic range sooner if you do this test before Coumadin therapy. However, they have not yet clearly
shown that this actually helps outcomes: that it helps the patients avoid bleeding or clotting complications. There is also the problem that warfarin is frequently initiated before the test results are available. This seems to dim enthusiasm for the test even further. Three large randomized clinical studies are pending which may make this test much more popular.

The other half to two thirds of the dosage variation is mostly due to foods and drugs. Because warfarin acts by eliminating active vitamin K, eating foods that contain a lot of vitamin K causes the body to require more warfarin to counteract the vitamin K. Foods contain a hugely variable amount of vitamin K, and therefore any change in diet can interfere with the INR. In addition, at least 22 herbal supplements and at least 65 drugs can interact with warfarin and change the INR. These include such common drugs as acetaminophen (Tylenol), any antibiotic, and even the flu vaccine. It is no wonder that doctors and pharmacists are required to constantly double check medications!

As a result of all these interactions, a large number of patients are not within the therapeutic INR range. For example in the US, only about half of patients are in the therapeutic range at any given time. Another third are too low and one in ten are too high; these patients would be at risk for bleeding or clotting problems.

The difficulties and tradeoffs of warfarin highlight the tradeoffs in using any powerful drug. The particular difficulty of being on warfarin anticoagulation is reflected in one patient’s blog after a recent article brought up the fact that the clot busting agent tPA (‘tissue plasminogen activator’) cannot be used for stroke patients who are also on warfarin. The blogger wrote, “For those of us who take Coumadin, it sometimes seems like we’re tip-toeing everyday through a minefield of potentially fatal risks and decisions. Some days I want to stick my head in the sand, ignore the risks and just live my life,” summing up exquisitely well the yin and yang of being on warfarin.

Back to our case subject, variations in diet or medications may have caused the INR to increase, or if the patient had recently started warfarin therapy she may have been given too high a dose for her genetics. The high INR may well have contributed to her death. However, always keep in mind that coincidence does not equal causality.

The Search for New Agents

So, warfarin is hard to dose, requires constant monitoring, is messed up by food, medicines, illnesses, etc., and can cause major and minor bleeding. Why is it still around?

Researchers have actually been searching for a better oral anticoagulant for more than 50 years. Development work has been intense since 1985, yet it was not until the dawn of the golden years of molecular pharmacology and molecular design that reliable, safe new oral agents were introduced.
In the last three years, however, three new oral agents have come on the scene:

1. Dabigatran, or Pradaxa, which acts as a direct inhibitor of thrombin, or factor IIa, was introduced in 2010.
2. Rivaroxaban, or Xarelto, which directly inhibits factor Xa (like low molecular weight heparins) was introduced in 2011.
3. Apixaban (Eliquis), another direct factor Xa inhibitor, was just introduced in 2012.

All of these act on the last few steps in the coagulation cascade. Dabigatran inhibits IIa (activated thrombin), the very last step before the fibrin clot. The other two inhibit Xa (activated factor ten), the step prior to that.
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These new agents are approved for a variety of indications or disorders:

- stroke prevention in atrial fibrillation (warfarin replacement)
- treatment of Deep Vein Thrombosis (heparin, LMWH, fondaparinux, warfarin replacement)
- prevention of DVT and PE (heparin, LMWH, fondaparinux, warfarin replacement)

The new agents have a variety of advantages over warfarin. First, for the majority of patients, the drugs do not require either adjustments in the dose or laboratory monitoring. For dabigatran (Pradaxa), for example, anyone with good renal function gets a 150 mg pill twice per day. There are no known interactions with foods and fewer interactions with other drugs.6

More importantly, clinical studies with fancy names like RE-LY, ROCKET-AF, and ARISTOTLE have so far shown that the drugs are somewhat safer, with significantly fewer serious intracranial and fatal bleeds. Not only that, they are somewhat more effective, with fewer strokes and fewer recurrent DVTs than patients on warfarin.7,8

The agents do have a number of disadvantages. Some of them are not readily absorbed from the gut. These drugs tend to accumulate in the GI tract, where they cause local anticoagulation effects, leading to increased gastrointestinal bleeding (and some abdominal pain). They also tend to cause increased risk of extracranial bleeding in elderly patients. Unfortunately, elderly patients, like our 82 year old case subject, are the very patients who could most benefit from the increased safety and effectiveness of the new drugs. Some studies have also shown that certain doses of these new agents cause an increase in myocardial infarcts, although the same studies show overall survival is better with the new drugs.9

There are no well-defined antidotes for the new drugs at this time. If a patient becomes over-anti-coagulated, it is a waiting game until the drug is cleared. They have, by design, very short half lives. If a patient misses even one or two doses, their risk of having a bad blood clot increases. The new agents are also more expensive, although the savings due to the lack of monitoring may somewhat level the costs. The new agents may be an improvement for selected patients but still need to prove themselves in longer term use.7

Case Study Two

A 62-year-old woman receiving the new factor IIa inhibitor Pradaxa (dabigatran) has an INR of 1.1, and the aPTT is mildly elevated. The physician inquires:

1. Are the PT and aPTT results consistent with dabigatran?
2. Do the elevations mean the patient is receiving enough dabigatran?
3. How can she get a level to see if the patient is receiving enough medication?
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The short answers are:

1. Yes
2. Not necessarily
3. There is no monitoring test!

What will the new agents do to PT and aPTT results and will the elevations show whether the patient is receiving enough medication?

Unfortunately, the answer is not straightforward. Dabigatran does not consistently elevate the INR/PT when at therapeutic levels, but may when large doses are taken. Dabigatran usually will cause a mild increase in aPTT, but the degree of elevation depends on the reagents and analyzer used. Sometimes, there is NO increase. Like so many other drug levels, the degree of elevation will also depend on how long before the blood draw the last dose was taken; there will be a “peak” level and a “trough” level.

*Rivaroxaban (Xarelto)* and *Apixaban (Eliquis)*, the factor Xa inhibitors (like low molecular weight heparin, remember?), typically elevate the PT more reliably than the aPTT. The INR does NOT help even out differences between reagents with these drugs. Again, the degree of elevation is not reliable or linear.

How can a dabigatran level be determined? There is no FDA-approved test available in the USA to measure levels of any of these agents. However, there are tests under development: for dabigatran, there is a modified thrombin time assay that does measure the levels very well, with a good linear relationship. Diagnostica Stago also has a “research use only” test available (the Ecarin clotting time, a snake-venom derived test). Tests are being developed for rivaroxaban and apixaban, but as they are the newest drugs, it may be some time before tests are available. An anti- Xa assay may be possible but will need to be validated with the two agents.

**Summary**

Because warfarin’s effects are so variable, new genetic testing has been developed to better predict response. New drugs, potential replacements for warfarin, have also been developed which appear to have some advantages over warfarin. The advantages may decrease or disappear in patients who are stable and usually have INRs in the therapeutic range while on warfarin. Implementation of these new oral anticoagulants will decrease the need for PT and aPTT testing over time, but it will take several years to determine the best agents for a given diagnosis. Monitoring for the new agents is not usually necessary, but could be helpful when patients present with bleeding, or if poisoning is suspected. No approved testing is currently available, but specific tests for each drug are likely to become available over the next several years.
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References


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