The who, when, and why of thrombophilia testing

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January 2024—Thrombophilia testing has been shown to be performed far more often than indicated in thromboembolic events, at significant cost to the patient and hospital.

“We’re constantly engaged with our clinicians about this topic,” said Marian Rollins-Raval, MD, MPH, professor of pathology at the University of New Mexico and medical director, TriCore special coagulation laboratory, in a CAP23 session on new guidelines for managing venous thromboembolism and how to reduce inappropriate test use. Then again, said co-presenter Neil Harris, MD, clinical professor of pathology, immunology, and laboratory medicine at the University of Florida, none of the recommendations on thrombophilia testing are “absolutely clear-cut,” and in some cases mitigating factors make testing necessary. Drs. Rollins-Raval and Harris are members of the CAP Hemostasis and Thrombosis Committee. (Dr. Harris’ term ended Dec. 31.)

In **Fig. 1** is the prevalence and thrombosis risk for selected thrombophilias. Many of the thrombophilias are relatively uncommon, occurring in less than one percent of patients who have a thrombotic event, though heterozygous factor V Leiden and antiphospholipid syndrome appear more frequently. Among the gain-of-function inherited thrombophilias, heterozygous factor V Leiden and heterozygous prothrombin gene mutation (PGM) have a lower relative risk of initial VTE than homozygous PGM and homozygous FVL. With the loss-of-function thrombophilias, the relative risk of initial VTE is significant. “So although they’re rare, we’re worried about them,” Dr. Rollins-Raval said.

After a patient presents with a thrombophilia, “we want to figure out if they should be on indefinite anticoagulation,” she said, and the risk of recurrent VTE can inform that decision. Patients with protein S deficiency, for instance, have a high relative risk of initial VTE, at 10-fold. But compared with the other thrombophilias, protein S deficiency doesn’t confer a higher relative risk of recurrent VTE.

Dr. Rollins-Raval asked the audience which of the following patients they would test for thrombophilia:

- A 76-year-old with a VTE after knee replacement surgery.
- A 55-year-old with a VTE after being hospitalized for pneumonia for a week.
- A neonate presenting with purpura fulminans.
- A 14-year-old starting oral contraceptive therapy.
- A 35-year-old male race car driver presenting to the emergency department with symptoms of a pulmonary embolism.
- A 60-year-old with atrial fibrillation admitted for a stroke.
- A woman who is currently 35 weeks pregnant with a strong family history of VTE.

“The two intended answers are the 55-year-old with a VTE after being hospitalized for pneumonia for a week—and this will become clearer with the guidelines that have recently come out—and the neonate presenting with purpura fulminans,” she said.

Fig. 1. VTE in common thrombophilia

	Thrombophilia	Prevalence (%)	Relative risk of initial VTE*	Relative risk of recurrent VTE*
Gain-of-function inherited thrombophilia	*Factor V Leiden (FVL) heterozygous*	2–7	4–6	1–2
	FVL homozygous	<1	7–19	2
	Prothrombin gene mutation (PGM) heterozygous	1–2	2–4	1–2
	PGM homozygous	<1	2–21	Uncertain
	Compound FVL & PGM heterozygote	<1	1–5	3
Loss-of-function inherited thrombophilia	Protein C deficiency	<1	10	2
	PS deficiency	<1	10	1
	AT deficiency	<1	10–30	3
	Antiphospholipid syndrome	2	7	2–7

*Rounded for clarity. Rollins-Raval M. VTE in common thrombophilia. Oral presentation at: College of American Pathologists annual meeting; Oct. 7–10, 2023; Chicago. Table adapted from Stevens SM, Woller SC, Bauer KA, et al. Guidance for the evaluation and treatment of hereditary and acquired thrombophilia. *J Thromb Thrombolysis.* 2016;41(1):154–164. doi:10.1007/s11239-015-1316-1. Licensed under CC BY 4.0.

Assay interference, patient labeling with its insurance and mental health ramifications, and the risks of anticoagulation are reasons not to test everyone. As is cost. Shen, et al., for example, estimated more than $1 million of wastage in a year on inappropriate testing at an academic teaching institution (Shen YM, et al. *PLoS ONE*. 2016;11[5]:e0155326). “When we think about it compared to the therapeutics, our testing seems minimal, but when it builds up for a time it could make a significant difference for a hospital,” Dr. Rollins-Raval said.

The American Society of Hematology last year offered 23 recommendations on thrombophilia testing and associated management (Middeldorp S, et al. *Blood Adv.* 2023;7[22]:7101–7138).

The guideline panel issued a strong recommendation against testing the general population before beginning combined oral contraceptives (COC). It offered conditional recommendations for thrombophilia testing in the following: patients with VTE associated with nonsurgical major transient risk factors or hormonal risk factors such as pregnancy, postpartum, or the use of COCs; patients with cerebral or splanchnic venous thrombosis, in settings where anticoagulation would otherwise be discontinued; individuals with a family history of antithrombin, protein C, or protein S deficiency when considering thromboprophylaxis for minor provoking risk factors and for guidance to avoid COCs or hormone replacement therapy; pregnant women with a family history of high-risk thrombophilia types; and patients with cancer at low or intermediate risk of thrombosis and with a family history of VTE. In all other scenarios considered, the panel provided conditional recommendations against testing for thrombophilia. Nearly all the recommendations were based on very low certainty in the evidence.

The recommendation to test patients with nonsurgical major transient risk factors—as in the example of the 55-year-old hospitalized with pneumonia for a week—departs from ASH’s prior guidance, Dr. Rollins-Raval said, which advised against testing in the setting of major transient risk factors, including prolonged immobility.

Dr. Rollins-

Raval

The American College of Chest Physicians' 2021 guideline provided one testing-related recommendation: Consider positive D-dimer in the decision to anticoagulate. The Anticoagulation Forum in 2016 advised against testing in the initial or primary phase of VTE treatment, in patients over 60 (for whom treatment is indefinite), in patients with provoked VTE (three weeks to six months of treatment), and in patients with arterial thrombosis or systemic embolism associated with known risk factors.

The Anticoagulation Forum recommended testing in patients under 50 with embolic strokes of unknown source and in rare thrombosis sites such as the cerebral vein and splanchnic vein without provoking risk factors, where paroxysmal nocturnal hemoglobinuria might be suspected. It also recommended testing in unexplained recurrent unprovoked thrombosis while on appropriate antithrombotic therapy, and when antiphospholipid syndrome is suspected. "Mostly what we're thinking about there is the choice of anticoagulation you might use," she said.

Patients with unprovoked VTE typically will be put on indefinite anticoagulation, though "every patient should be a discussion," she said. "Think about the race car driver. You might have wanted to test him when he presented to the emergency department, but do you want to put a 35-year-old race car driver on indefinite anticoagulation? He could be at risk more for bleeding than clotting."

Thrombophilia testing isn't recommended in VTE provoked by surgery because the risk of recurrence is low.

For interference reasons, it also should be avoided in patients in the acute stage of thrombosis, in the initial phase of VTE treatment, and in patients who are pregnant or on anticoagulation.

Though testing shouldn't be done in the acute setting of VTE and/or in the hospitalized patient, "one thing you could consider testing in the ED or as they come in would be D-dimer," Dr. Rollins-Raval said, to exclude VTE in patients who present with symptoms but have a low clinical probability score. "You want to make sure the assay you're using is approved to do that." The test should not be used to rule in VTE.

"Another thing you might consider inpatient is testing for antiphospholipid syndrome, if they present with signs of catastrophic antiphospholipid syndrome." If the patient has cerebral venous sinus thrombosis or splanchnic vein VTE, "you could think about testing for paroxysmal nocturnal hemoglobinuria if they have cytopenia and hemolysis, or you could think about testing for a myeloproliferative disorder if the patient has cytosis." And patients on heparin with evidence of thrombocytopenia may need testing for heparin-induced thrombocytopenia.

But in many cases, no other inpatient testing is recommended because of possible interference, particularly on the functional assays. "You could have consumption of the coagulation factors in the clot," she said, or an increase in acute phase reactants (factor VIII, fibrinogen, or C-reactive protein). Underlying medical conditions such as nephrotic syndrome, infection, disseminated intravascular coagulation, or liver impairment also can cause interferences, as can anticoagulants.

In addition, inpatients may be lost to follow-up. "For us, one of the challenges we have is factor V Leiden testing ordered in the hospital on an inpatient," she said. With patients typically discharged before the test result is available, "often we didn't have follow-up to let them know they had that diagnosis." Confirmatory testing, too, is sometimes required but not performed if the patient doesn't return to that setting. For example, if a hospitalized patient has nephrotic syndrome and a low protein S, confirmatory testing would determine if the low protein S is because of the nephrotic syndrome or because there's an underlying deficiency as well. "But that may not be performed."

All the functional assays for thrombophilia could potentially be affected by anticoagulation or other factors, Dr. Rollins-Raval said. Factor V Leiden and prothrombin gene mutation shouldn't be affected. "You could also test for solid-phase/immunoassay-based antiphospholipid antibodies, although we know those are sometimes falsely

elevated with inflammatory conditions. But theoretically those should be accurate, even on an inpatient."

Dr. Rollins-Raval shared how two institutions—one a public academic teaching hospital, the other a private hospital—reduced inpatient testing for thrombophilia.

Fig. 2. Anticoagulant effects on coagulation assays (TriCore coagulation laboratory)

Assay	Unfractionated heparin	Low-molecular-weight heparin and fondaparinux	Vitamin K antagonists (i.e. warfarin)	Direct factor Xa inhibitors (i.e. Xarelto, Eliquis, edoxaban, Savaysa)	Direct thrombin inhibitors (i.e. Pradaxa, argatroban, bivalirudin)
Antithrombin activity, FXa based	no interference up to 4.0 IU/mL	no effect	no effect	falsely high	no effect
Protein C activity, chromogenic	no effect	no effect	decreased	no effect	no effect
Free protein S antigen	no effect	no effect	decreased	no effect	no effect
LA tests (including DRVVT and LA PTT)	unaffected by HepXa levels <1.00 IU/mL	unaffected by HepXa levels <1.00 IU/mL	Best when INR <1.50	False-positive results—testing will not be performed	False-positive results—testing will not be performed
Beta-2-glycoprotein-1 antibodies	no effect	no effect	no effect	no effect	no effect
Cardiolipin antibodies	no effect	no effect	no effect	no effect	no effect
Factor V Leiden gene mutation	no effect	no effect	no effect	no effect	no effect
Factor II prothrombin gene mutation	no effect	no effect	no effect	no effect	no effect

At the University of New Mexico Hospital, of 403 inpatients tested over several years before the intervention was implemented, 49 percent were on an anticoagulant at the time of testing (Elmaoued AA, et al. Abstract No. 320 presented at: Western States Conference; May 20–22, 2019; San Diego). Of those, about a hundred were on low-molecular-weight heparin, with the rest on apixaban, rivaroxaban, fondaparinux, and others. Two hundred of the patients had a thrombotic event while in the hospital; of those, 84 percent (167) were tested in the acute phase, and 68 percent (135) had a provoked thrombotic event. Ninety percent of the patients had genetic testing, and of those, six percent (20) had unnecessary repeat testing. (The lab has since instituted a review of prior testing and cancels orders for repeat genetic testing or submits the prior results.) Conversely, of the 96 percent tested for antiphospholipid syndrome, 72 percent (74) of those who tested positive did not receive the recommended repeat testing within 12 weeks. Forty patients with an active or recent pregnancy were tested.

Of the 403 patients tested, only 30 were tested appropriately, Dr. Rollins-Raval said. "And those were for an autoimmune workup and could have been done in the outpatient setting." Most of the inpatient obstetric antiphospholipid antibody testing was done inappropriately due to recent pregnancy, and all inpatient heritable thrombophilia testing was done inappropriately.

A clinician champion spearheaded an initiative to restrict thrombophilia ordering (work that predated Dr. Rollins-Raval joining UNM), in which the single hypercoagulation panel that included all thrombophilia testing and could be ordered without restriction was replaced in the electronic health record with two separate panels: acquired thrombophilia, which includes the antiphospholipid antibody panel and remains unrestricted, and inherited

thrombophilia, which is partially restricted for inpatient adults. All orders for protein C activity, free protein S antigen, factor V Leiden, and prothrombin gene mutation are subject to approval. Though the antithrombin test has two variants that are not restricted, they're listed under separate orders in the EHR—AT3-ECMO and AT3-Pediatric—rather than under antithrombin thrombophilia. "So people have to know what they're looking for," she said. OB-GYN and pediatrics elected not to participate in the intervention.

Total inpatient orders for factor V Leiden decreased 95 percent, from 170 in 2015 (pre-intervention) to eight in 2022, all of which came from pediatrics. But pediatric orders, too, declined. "We had about 30 orders in 2015 on inpatient pediatrics," she said, compared with the eight in 2022. Outpatient orders declined as well, from 300 in 2015 to under 150 in 2022, the result of education around appropriate test use.

In the intervention's earlier days, a notice in the EHR advised test orderers to call an antithrombosis provider for a consultation or, in the off hours, the inpatient antithrombosis service. "When I got there, they were overwhelmed, so we pushed it to the pathology resident on call," where it remains today.

At the private hospital, the pathology department led an initiative to reduce inappropriate thrombophilia testing, with a second signature requirement for all inpatient, ED, and urgent care thrombophilia orders built into the EHR. Ordering physicians are shown a set of process instructions explaining that if thrombophilia testing is indicated, it should be done only in the outpatient setting, ideally with specialist guidance. A physician who still wishes to place the order is directed to call the special coagulation pathologist for a consultation and co-signature. Antiphospholipid antibodies and antithrombin testing can be ordered without the second signature, and all orders from hematology and oncology remain unrestricted.

In 2016, before the intervention, 347 inpatient factor V Leiden tests were resulted, for a total cost of $34,700. After the intervention, in 2022, 15 FVL tests were resulted, for $1,500. Pathologists canceled 43 factor V Leiden test orders in 2022. The number of outpatient tests increased, from 95 in 2016 to 176 in 2022.

"The ongoing discussion between our providers and pathology in the private hospital has helped over time to decrease these orders," Dr. Rollins-Raval said. "It's been more challenging in the academic hospital, given the shifting of trainees and new faculty, but it's something we're constantly ready to discuss with our providers and it seems to be helping."

In **Fig. 2** is an educational aid summarizing the effects of the anticoagulants on the coagulation assays. "We have this available for all providers on our directory of services, and we refer them to this if they try to test someone when they're on an anticoagulant," she said.

Dr. Harris' institution doesn't restrict inpatient thrombophilia ordering. "So if we suspect anticoagulant use, we have to screen for it, and this has become problematic with the technologist shortage," he said.

Dr. Harris

"One of the things you can do to look for anticoagulants is run a PT/INR to check for warfarin," he continued. For anti-Xa direct oral anticoagulants and heparin, "you can do an anti-Xa assay calibrated with unfractionated heparin or hybrid heparin curve." And to exclude direct thrombin inhibitors, "you can perform a thrombin time," which will screen for the oral direct thrombin inhibitors such as dabigatran and the intravenous direct thrombin inhibitors such as bivalirudin. "And we've seen a lot of bivalirudin use at our institution." Thrombin time also can be used to

screen for heparin.

“As a rough guide for the anti-Xa DOACs,” he said, “you may see a prolongation of the PT/INR, more so with rivaroxaban than with apixaban.” The anti-Xa assay will be positive (>0.3 U/mL) using an unfractionated heparin or hybrid calibration curve. “If it’s a DOAC, that usually means it’s higher than about 35 nanograms per mol, which is the low trough for the DOACs.” The thrombin time should be normal.

When direct thrombin inhibitors are onboard, PT/INR may be prolonged but often can be normal. The anti-Xa assay will be normal. Thrombin time will be significantly prolonged, he said.

If the patient is on LMWH, the PT/INR will typically be normal, but the anti-Xa assay will show increased activity and the thrombin time will be prolonged. And with warfarin, “the PT/INR should be prolonged, but it should correct on the one-to-one mix.” The anti-Xa activity will be negative or normal, as will the thrombin time.

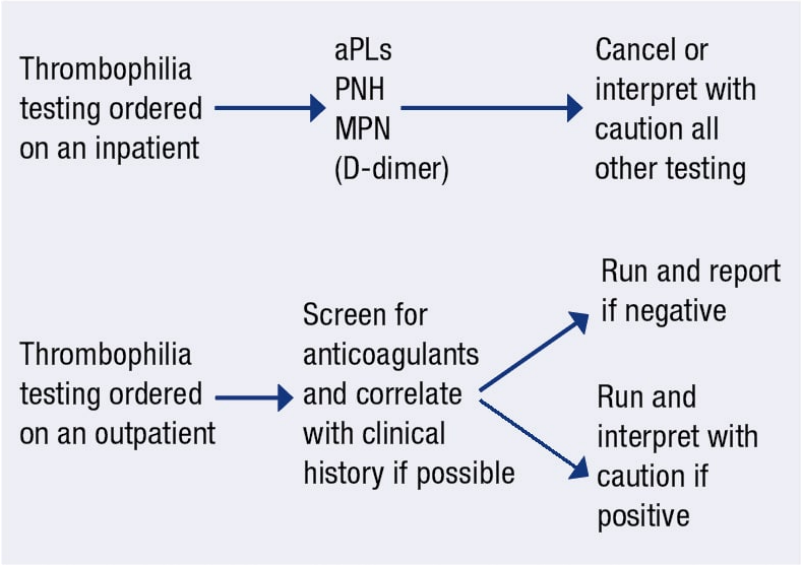
In all cases, Dr. Harris noted, it’s critical to review the chart.

Many of the thrombophilia assays can be falsely elevated by anticoagulants such as rivaroxaban or high concentrations of heparin, he said. If a patient is on rivaroxaban, for example, and the Xa-based antithrombin activity is normal, the medication may have artifactually elevated the antithrombin activity, normalizing the level.

Thrombophilia testing, then, should be done after the patient has completed the initial treatment course for VTE and is no longer on anticoagulation. Warfarin should be stopped two weeks before testing. DOACs should be stopped 48 hours before testing, and earlier in patients with renal impairment. “You can test with unfractionated heparin provided it’s in the therapeutic range, preferably well below 0.7 units per milliliter,” he said. And patients on LMWH can be tested, but samples should be drawn at least 12 hours after the last dose. “A lot of our hematologists do switch patients from DOACs to enoxaparin in preparation for testing if they need to test in that period before the three months is up.”

Dr. Harris shared a general laboratory algorithm for thrombophilia testing (**Fig. 3**).

Fig. 3. Approach to thrombophilia testing—laboratory algorithm



Thrombophilia testing can be ordered on an inpatient if the patient is suspected of having antiphospholipid syndrome, he said, and particularly catastrophic antiphospholipid syndrome, “although I say that with some reservation because if the patient is not suspected of having catastrophic antiphospholipid syndrome, you have to be aware that the acute phase reaction may cause a transient positive lupus anticoagulant and transient positive anticardiolipin antibodies.” If paroxysmal nocturnal hemoglobinuria is suspected, flow cytometric analysis could be

done, and if myeloproliferative neoplasm is suspected, “the testing would involve further hematology workup, including a bone marrow examination.” In all other cases, inpatient testing should be canceled or interpreted with caution.

In the outpatient setting, “you should screen for anticoagulants and correlate with the clinical history,” he said. If no anticoagulants are onboard, run the test and report it. If the patient is on an anticoagulant, “some labs may cancel; other labs may run and interpret with caution if positive. We tend to adopt that approach.” For example, he said, warfarin may cause a false-positive or false-negative anti-phospholipid test.

“The best thing is to try to avoid the anticoagulants if possible when testing.”

Charna Albert is CAP TODAY *associate contributing editor.*