Hello. I’m Richard Marlar from the University of Oklahoma Health Sciences Center and the Oklahoma City VA Medical Center. Today I’m going to talk about a cost-effective evaluation for thrombophilia.

Initially, I will talk about thrombophilia and thromboembolic disease, and then we’ll talk about thrombophilia as a multifactorial genetic disease. Then we’ll talk about the thrombophilic factors such as protein C, factor V Leiden and others, and then we’ll finish the session talking about how to effectively evaluate for thrombophilia.
In the next slide, this just reviews the incidence of thromboembolic disease in the United States. Thromboembolism, both arterial and venous, are the most common cause of death in North America. Between 5,000,000 and 6,000,000 people per year develop thrombosis, and 300,000 to 500,000 deaths per year are caused by both arterial and venous thrombosis.

Venous thrombosis is a complication of a wide spectrum of diseases including cancer, diabetes, and other conditions. The incidence of preventable venous thromboembolic disease is about a half a million cases per year.

Thrombophilia is a portion of venous thromboembolism. Its definition is a set of genetic abnormalities and/or acquired conditions which increase the risk for an individual for developing thromboembolic disease. It’s a broad, general definition, but one which we can work with.
Unfortunately, there are some inconsistencies within thrombophilia. There is a lack of correlation between the genotype such as protein C deficiency and the resulting phenotype, venous thrombosis. We find that some families have no known genetic risk factor, yet they’re symptomatic, whereas other families have known deficiencies, but their whole family is asymptomatic.

How can we account for all of these inconsistencies with a model for thrombophilia? And we’ll discuss where that is at this point. But before we start the discussion on the model for thrombophilia, we need to talk about some of the concepts.
The first concept that we need to discuss is risk factor. A risk factor is any condition, whether it’s genetic or acquired, which predisposes an individual to a greater than normal chance for developing a thrombosis. Risk potential is actually a capacity of the risk factor to contribute to thrombosis.

Some risk factors have a low risk potential such as factor VIII elevation, and some have a high risk potential, like antithrombin deficiency. We measure that in relative risk. That’s a genetic term that actually measures the extent of the risk factor potential.

And finally cooperativeness, that is the interaction of two risk factors which increases the total risk to the patient, which is greater than the sum of the two individual risk factors. In the next slide, we show the three main types of risk factors that are present in thrombophilia.

We have physiologic risk factors which are ones that we cannot change, such as age and gender. We have genetic, which can be hemostatic or nonhemostatic risk factors that actually cause increased risks. And finally, we have acquired risk factors which can be behavioral or pathologic, and we’ll discuss these in more detail.
Talking about the physiologic risk factors, this slide shows the relationship of thrombosis with age as well as the percent of thrombosis in males and females. As you can see with each increasing decade in life, the incidence of thrombosis per 100,000 individuals increases significantly, and if you look at the gender proportion, 60% of the males have thrombosis compared to about 30%. So there is a discrepancy until you reach menopause, and then in the older population, they become similar.

Genetic factors, we have of course the established factors, which are shown here in this slide, we have protein C, protein S, antithrombin, factor V Leiden, factor II, the mutation, the prothrombin mutation that’s 20210, hyperhomocysteinemia, and an elevation of factor VIII. Those are established. We have a number of them that are probable risk factors or mild risk factors or suspected risk factors.
They include dysfibrinogenemia, the PLA-2 on platelets, factor XIII which turns out to be protective for that polymorphism, and elevation of factor XI just to name a few. But there are a number of them we still don’t know yet. Acquired risk factors, in the next slide, can be subdivided into a variety of different classes.

They include environmental, pathological, behavioral, and physical. And as you can see from this slide, there are a number of them. The pathological ones of course, are the antiphospholipid syndrome, cancer, chronic inflammation, atrial fibrillation, and a previous venous thrombosis.

Behavioral, things that we can actually change, are smoking, poor diet, no exercise, and the birth control pill. And there are some that we can minimize or not change at all. So we have looked at physiologic, genetic, and acquired. Now how do we put all this stuff together?

Well, let me show you some more data here to put it really all together. The factors here that are genetic; this slide shows the incidence and the risk that has been associated, and this was all taken from the literature. As you can see, the incidence of antithrombin, protein C, and protein S deficiency is pretty rare.
However, the relative risk is fairly high. Factor V Leiden and the prothrombin mutations are pretty prominent in the Caucasian population. But their risk is fairly low. And then you start looking at things that are graduated such as homocysteine, elevated factor VIII, and others, and they have a fairly high incidence, but again the relative risk is low.

As you can see, we have high risk and low risk. Here’s the slide of showing some data that we have put together, showing several factors which are minor but do contribute. Blood type. When you consider patients with type AB and B have a higher risk for thrombosis, almost a two-fold increase compared to those with type O.

We also see hs-CRP, C-reactive protein, have a higher risk. Again it depends on how much CRP is present in the patient’s plasma. And, as I said, factor XIII is protective, and you have a risk of less than one in that case, if you have that polymorphism.

Now if we try to put together this multi-hit hypothesis, what we have with some of the factors is 1, many hereditary and acquired risk factors are present, probably 20 to 50, most are relatively small in their risk potential; however, if you have more than two, or two or more, you have a greatly increased risk potential in these individuals.
What are we talking about in this cooperativeness? Well, here’s an example of looking at factor V Leiden and the prothrombin mutation. And in the slide, if it’s N stands for normal and A stands for the heterozygote state, if you have normal factor V and normal prothrombin, you have a relative risk of 1.

If you’re abnormal for factor V Leiden, but normal for prothrombin, you have a risk of 1.8. If you have an abnormal prothrombin 20210, you have a risk of 2.5; however, if you have both, you don’t have a risk of 4, you have a risk of almost 23 times greater. So having two is a much greater risk than having each one individually.

So it is a cooperativeness between these two risk factors. Here’s another example of looking at several of the common genetic factors with the oral contraceptive. If you are taking the birth control pill, but have no genetic risk, you have a 3.6-fold increased risk for developing thrombosis.
However, if you have factor V Leiden and are taking the birth control pill, you have a 24-fold increased risk, and a 16-fold risk if you’re taking the birth control pill and you have prothrombin 20210.

Now if you consider patients taking birth control pills who have a protein C deficiency, they have a greater than 80-fold chance of developing thrombosis compared to a normal individual. So an acquired factor and a genetic risk factor can give a drastically increased relative risk.

Here if we take a number of polymorphisms, they have very minor risks by themselves, but if you have all of them, you have a greater than 20-fold chance of developing thrombosis. So you take these multiple factors, and you put them together as, in one patient, they have a significantly increased risk compared to having these individually.

Here’s the model that we have used and made to attempt to try to accommodate all of these known facts. If you take the genetic, acquired, and physiologic risk factors, and you take them for the whole individual, you get a total relative risk.
If that risk is above some threshold, which we don’t know yet what that is, and you have a stimulus such as injury to the vessel or surgery, you will develop thrombosis; however, if that relative risk is below the threshold, and you still have the stimulus, you do not develop thrombosis.

But you must have a stimulus, whether above or below that threshold to develop that thrombotic; that is the basic model for the multi-hit thrombophilia paradigm that we have. Here’s an example of how it fits together. As we talked about, increasing of age along the X-axis, you see an increased risk for developing thrombosis. On the Y-axis here, we have increased relative risk.

In the normal individual, you see a slight increase in that risk. However, if a patient has, say, prothrombin 20210 mutation, that risk is significantly increased compared to normal; however, it does not go above the threshold for developing venous thrombosis.

Now in a patient who has taken the birth control pill with the prothrombin mutation, we see a spike in the relative risk; however, it is not above that threshold to thrombosis. And then later on, this woman gets pregnant, and again, the risk is increased with pregnancy, but again, not above the threshold.

However, if during pregnancy, or right after pregnancy, this woman develops an antiphospholipid syndrome, the relative risk goes above the threshold and with any stimulus, she develops thrombosis. If you look at this in a normal individual, even in the 80s and 90s years of age will not develop thrombosis.
However, patients with a prothrombin mutation or a factor V Leiden will develop thrombosis. Now, what are we going to do with this model? Eventually, we’re going to be able to assess all of these polymorphisms and these mutations, and basically this slide summarizes what we’re going to end up doing.

**Title: Laboratory Evaluation of Thrombophilia**

Acquired factors will be ascertained by the clinical provider, and we’ll also use a protein array method to determine multiple factors such as factor VIII or hs-CRP or homocysteine or other factors in that order, and also the polymorphisms will be ascertained with gene chip technology.

So using both of those which are very inexpensive, we will be able to find out all the risk factors of this individual and be able to interpret their interaction in a computer-derived model and be able to provide to the clinical provider, the overall thrombotic risk.

This will be important if the patient is going to surgery or being put on the birth control pill or hormonal replacement therapy. So with that model, we need to change gears, and we’ll talk about the established risk factors. This slide shows the naturally occurring anticoagulants.

**Title: Cost Effective Evaluation for Thrombophilia - Established Risk Factors**
As you can see, we have antithrombin, protein C, protein S, TFPI tissue factor pathway inhibitor, and heparin cofactor II are the main four that we know of. Unfortunately we don’t know of deficiencies of TFPI or really of heparin cofactor II. The big ones are antithrombin, protein C, and protein S.

We also have elevated factor VIII and factor V Leiden, and elevated factor XI also in this slide. Well, let’s talk about the common ones first.

Antithrombin deficiency. Antithrombin was the first reported inherited thrombophilic state. It was reported in the 1950s.

Title: Antithrombin Deficiency

It is the most severe inherited disorder with a very high relative risk; however, fortunately it’s relatively uncommon. It’s less than 1% for those patients who have had their first venous thrombosis. It is an autosomal-dominant inheritance pattern, in other words, heterozygotes are symptomatic.

Normally we see levels between 30 and 60%. We’ve never seen a patient with less than 1% or 0%. I think it’s not compatible with life. The onset of first thrombotic event is usually in the early adult years, 15 to 30 years of age. There are inherited and acquired deficiencies.

We see decreased levels of antithrombin in pregnancy, the birth control pill, and hormonal replacement therapy. We measure this
antithrombin usually using a chromogenic assay, that’s the standard method. We can also measure it antigenically.

Now for the protein C and the protein S system, we look at the protein C pathway. This is the typical pattern that we see or the pathway that we see. The, on the left hand side, we have the extrinsic and intrinsic system of coagulation showing that factor VIII and factor V are very important in thrombin generation. The majority of thrombin develops a fibrin clot.

Title: Protein C Pathway Mechanism

![Protein C Pathway Mechanism](image)

Some of that thrombin, however, goes downstream in the blood vessel and binds to the thrombomodulin, the TM, on the surface of the endothelium. And once that complex is formed, protein C which circulates in plasma, is rapidly converted to activated protein C.

Activated protein C in the presence of protein S rapidly inactivates factor Va and factor VIIIa to an inactive product, turning off the coagulation system. APC is inhibited by several inhibitors in plasma. It also has a fibrinolytic component.

Now if we talk about protein C deficiency, we can see by this slide, that we also have inherited and acquired deficiencies. Acquired deficiencies occur in liver disease, oral anticoagulant therapy, and several other conditions. The onset in the inherited disorders usually again are between the ages of 15 and 50, in the younger adult years.

Title: Protein C Deficiency

![Protein C Deficiency](image)

It’s autosomal-dominant; however, the penetrance is variable. We normally see in the heterozygotes, which is the common ones we find, is a level between 30 and 60%. We have multiple gene defects, it’s not a single polymorphism, but we have multiple gene abnormalities.
You can assay protein C by a clotting assay, a chromogenic assay, or an antigenic assay. We recommend clotting assays, as there you will see the majority of the defects. Antigenic does not measure function, and will miss the type IIIs, and chromogenics may also miss some as well.

Protein S deficiency is a little more complex, but again, we have inherited disorders, again, due to multiple gene defects such as we see in protein C. It’s autosomal-dominant, with incomplete inheritance, excuse me, incomplete penetrance such as we see with protein C deficiency.

Title: Protein S Deficiency

Again, age of onset is similar to protein C, but acquired deficiencies occur in a number of different cases. We see in pregnancy, especially third trimester, protein S levels that are very close to undetectable. Also in patients on the birth control pill and with HRT, the levels will decrease.

Inflammation and acute thrombosis will also decrease protein S levels. Well, and I’m going to show you some data of this in a minute, but we can also measure protein S by a functional assay, but also by a free protein S antigen assay or a total protein S antigen assay.

The functional protein S varies depending on the amount of free protein S, and I’ll show you that in the next slide. It correlates with physiologic function; however, the big problem is, is that we can see erroneous results due to a variety of artifacts, which I’ll discuss in a minute.

This slide shows how protein appears, protein S appears in plasma. It is either free, unbound, or it is bound to the complement protein, C4b binding protein. About 60% is bound, 40% free in the average normal individual. But remember, only the free protein S is the active protein S.
In the laboratory, as I said, we can measure activity, free protein S antigen, or both total which is free and bound together. The normal ranges are very similar when you compare it to a normal plasma pool. The most reliable assay to perform is the protein S free antigen assay.

And this data on this slide shows why there are assay inconsistencies with protein S. We looked at over 850 patients who had been sent to us as a reference laboratory for protein S activity. And about 11% of those patients had abnormal low protein S activities compared to the free protein S.
This was apparently due to the elevated factor VIII levels, cold activation of plasma, and other causes which we were not familiar with. When we repeated the majority of these low samples with new samples that we obtained, 90% of the low protein S activities now compared with the free protein S antigen levels and they were normal.

Also we found in a number of cases, that the protein S activity levels were significantly elevated in some cases with normal total protein S, normal free protein S, we had protein S activities of 800 to 1200% of normal, which was unknown in the cause for us.

We found that free protein S sometimes is misdiagnosis of a deficiency in about 1 to 45 patients with protein S deficiency. It’s fairly rare, but it’s a good first order test, and total protein S does not detect the interaction between C4b binding protein and protein S. So we have no idea.

In some patients, we have a deficiency, but others we do not. So based on this data, we recommend that you use a free protein S antigen assay as your initial screening assay, and then go back if it is low, and do the protein S activity and the total protein S antigen.

Now let’s switch to the most common disorder that we’ve seen so far, and that is APC resistance. APC resistance is the inability of a patient’s plasma to be inhibited by activated protein C. If we add activated protein C to a patient’s plasma, to a normal patient’s plasma, we should go from 30 seconds for the PTT up to over 100 seconds for the PTT.
However, in patients with APC resistance, that 30 seconds will only go to 45 or 50 seconds. That’s the resistance that we see in these patients. Approximately 30% of these patients with this resistance are due to what is known as factor V Leiden, 60% are due to an acquired cause, and I’ll list those in a minute.

The remaining 10% appear to be genetically based, but we don’t know what the defect is. When we compared the relative risk for the development of thrombosis in these three groups, we found that the relative risk for factor V Leiden was only 2.4, but the relative risk in the acquired causes in about 60% of the cases was actually 3.2, significantly higher than for factor V Leiden.

So it is important that you test for APC resistance. Factor V Leiden is one of the major causes of APC resistance. It turns out that factor V Leiden is a mutation in the activated protein C cleavage site in the factor V molecule.

**Title: Factor V Leiden and APC Resistance Genetic Mechanism**

- **Molecular Cause**
  - Mutation of an APC cleavage site in Factor V
- **Mutation Site:**
  - Amino acid #506 is Arg and mutated to Gin
- **DNA and protein sequence:**
  - Amino Acid: Arg$_{506}$ \[\rightarrow\] Gin$_{506}$
  - Nucleotide: CGA \[\rightarrow\] CAA
- **Molecular Function:**
  - APC cleaves Arg-Gly bond.
  - If mutated to Gin, then bond not cleaved.
  - FVa remains active generating more fibrin clot.

Meaning that the factor V is not inactivated as rapidly as in a normal individual. It turns out that it’s a mutation in amino acid 506, which is normally an arginine, but has been mutated to a glutamine.

What this basically means is that activated protein C does not inactivate factor V. What type of assay should we be doing for APC resistance if we want to determine whether it’s factor V Leiden or not factor V Leiden due to an acquired cause? Well basically, there’s a variety of assays out there.

**Title: APC-Resistance Assays**

- **Plasma Based Tests:**
  - Numerous variations of the APC-R assay
  - Non-specific for general inhibition of coagulation
  - Specific for Factor V$_{Leiden}$
  - **Specific tests for Factor V$_{Leiden}$ utilizes:**
    - Factor V deficient plasma
    - Non-APTT based test (RVVT)
- **Genetic Based Tests:**
  - DNA and PCR
  - FRET technology
  - Gene Chip based
There are nonspecific assays for the general inhibition of APC resistance, and then there’s ones that are specific for factor V Leiden. Some use APC resistance assays with an aPTT-based assay or a RVV, or Russell’s viper venom assay. Some use factor V deficient plasma, others do not. We can test APC resistance by a variety of methods. We can also test for factor V Leiden using PCR and DNA technology.

In the nonspecific assay, which is what I’m going to recommend that you do, we use an aPTT-based assay without adding factor V-deficient plasma. This picks up not only the factor V Leiden patients, but it also picks up those patients that have acquired abnormalities of APC resistance.

Title: APC-Resistance Assays - Non-Specific Assay

![APC-Resistance Assays - Non-Specific Assay](image)

Basically, the causes of an abnormal APC resistance besides factor V Leiden are elevated coagulation factors such as factor VIII, that’s the most common; defects in the protein C system such as protein S and that again is the main one; lupus anticoagulant; pregnancy, and HRT and birth control pill; and the thrombotic process.

All of these are elevated risk factors for thrombosis, and they all give you abnormal APC resistance assays without factor V-deficient plasma. So that would be our recommendation.

Now switching to the next common one, that is the prothrombin 20210 mutation. It’s a polymorphism at a base pair in the gene of prothrombin at 20210 or twenty thousand two hundred and ten base pairs from the start. It is in the untranslated region and it appears to increase the prothrombin levels in the plasma.
Title: Prothrombin-UT 20210 Mutation

- Polymorphism at bp 20210 in 3’ UT
- Increases Prothrombin Levels (?)
- Mechanism: longer surviving mRNA
- Genetic Abnormality
  - Heterozygote and Homozygote
- Assay: DNA based only
- Relative Risk: 2.0

Its mechanism is probably causing longer survival of the message RNA. We have seen heterozygotes and homozygotes. Homozygotes are fairly rare. The relative risk is about 2. And the only way we can assay for the prothrombin 20210 is with a DNA-based assay. There are a variety out there on the market.

Turns out this mutation is not in the translated portion. If you look at this slide, in the top row with the single letters are the end of the prothrombin gene, the three asterisks are the stop codon, and the continued in second, third, fourth lines are all of the base pairs that come after the gene stops, excuse me, after the translated gene stops.

Title: Prothrombin-20210 Gene Polymorphism

As you can see at the G towards the end of the third line, you have a red G. That is mutated to an A in the polymorphism. So it is not in the translated region, and it appears to cause the problem.

In this slide, we show what happens with prothrombin levels when we look at normal individuals, that is without the mutation that is in the red graphs, those are quartiles of the normal range; and in the yellow are those patients who have the mutation and what their prothrombin levels is.
You can see that greater than 90% of the patients with factor II mutation have prothrombin levels higher than 115%, so they're on the elevated side. When we look at the incidence of factor V Leiden and prothrombin in US population, what we see here is that it's mostly a Caucasian disease.

If we're going to stratify and test based on ethnicity, then we will have to take this into account. But at this point, in the American population of African Americans, Asian Americans, and native Americans, and the Hispanic population, we do have a slight chance of developing or having this mutation.

Factor VIII is a new thrombotic risk that has been started to be evaluated commonplace, and as you can see again, as you increase the levels of factor VIII, the relative risk increases. If you have greater than 180% factor VIII, you have a relative risk of almost 4. It appears that it can be inherited, but it can also be an acute phase reactant.
Title: Thrombotic Risk for Factor VIII Levels

So if you have an elevated factor VIII level, you need to repeat that in a couple of weeks to see whether it's an acute phase or whether it appears to be inherited. Homocysteine is another one that we’re testing. Homocysteine is an amino acid. It's involved in the metabolic regulation of methionine and cysteine.

Title: Homocysteine Biochemistry and Pathophysiology

Elevated levels causes perturbations in the endothelial cell. We reduce the antithrombotic response with protein C and protein S and thrombomodulin, but we also increase the prothrombotic response. We increase the levels of tissue factor on the surface of the endothelial cell.

We can decrease homocysteine levels with the vitamins, folic acid, and B6 and B12. There are genetic and acquired causes of elevated homocysteine levels. This slide shows how all of these different factors can affect homocysteine levels.
Title: Pathogenesis of Hyperhomocysteinemia

We can have genetic factors, but we have a lot of acquired factors such as drugs, age increases it, nutrition, vitamin status, can all increase homocysteine levels, which increases cellular damage which causes thrombosis. We can measure homocysteine levels in plasma, and it turns out it's an independent risk factor for venous thrombosis. Again, as you increase the levels, the relative risk increases.

Title: Evaluated Homocysteine Levels and Venous Thrombosis

It goes up to 3.4 relative risk. Homocysteine levels are increased with age, but also the risk with age increases. It appears that the endothelial cell is not able to process homocysteine as well when you're older. One important point is that we have found no correlation of homocysteine levels with either of the MTHFR mutations, 677 or 1298.

So in other words, if you're homozygote or normal for MTHFR, it has no bearing on your homocysteine level nor on whether you will develop venous thrombosis. Next, we want to talk about antiphospholipid syndrome and thrombotic risk.
This is a lot of detail to talk about in one slide, however, but basically the ISTH subcommittee recommends that, the following protocol. You perform an aPTT with a lupus anticoagulant-sensitive reagent, you perform mixing studies, and then you perform three lupus-specific tests when you add back phospholipid.

You can either use a platelet neutralization procedure, an RVVT, or hexagonal phospholipid test. One of those need to be positive for antiphospholipid syndrome. Also, you can test antcardiolipin, antiphospholipid antibodies and anti-beta-2 glycoprotein-1 to determine whether the patient has antiphospholipid syndrome.

If any of those are positive and remain positive after testing again in four to six weeks, that patient has a lupus anticoagulant or antiphospholipid syndrome. What is the prevalence of these abnormalities in patients with venous thrombosis? We see this in this slide here.

In antithrombin, protein C and protein S are fairly rare. APC resistance is fairly common. However, factor V Leiden is not as common as APC resistance. Homocysteine and antiphospholipid syndrome are fairly common again, and we still have 20 to 40% that are unknown, that we have no diagnosis for at this point.
Okay. How do we evaluate patients who have a thrombophilia? This slide shows how we would go about evaluating it, and some of the important points that we need to consider. Evaluations for thrombophilia are complex, they’re variable, they change over time, and they will continue to change.

Many of these evaluations are provider preferences, hospital laboratory demands on how you test and what you test. Workups can be from anywhere from $600 to over $2000, and we must decide what is the most cost-effective way to do that. Should we stratify, either by age, gender or ethnicity? In our hands, we stratify it by age.

Should we be performing thrombophilia testing? Well, does the patient’s history justify it? Was there some other cause for this thrombotic event in an older individual? Are the tests that we’re going to use ordered correctly at the appropriate time?
Title: Should a Laboratory Evaluation for Thrombophilia Be Performed?

Should a Laboratory Evaluation for Thrombophilia Be Performed?

- Does the patient’s history justify an evaluation?
- Are the tests correctly ordered to properly evaluate the patient?
- Are there underlying therapeutic, pathologic or physiologic conditions that will interfere with the interpretations of test results?
- Will the results benefit the patient or family?

Are there therapeutic, pathologic or physiologic conditions that we need to take into account that’s going to interfere with interpretation, and will the results benefit the patient? Why do we need to evaluate for thrombophilia? One, we need to educate the asymptomatic individual.

Title: Why Evaluate for Thrombophilia

Why Evaluate for Thrombophilia

- Educate asymptomatic individuals
- Provide genetic counseling
- Provide prophylactic therapy for high risk
- More appropriate anticoagulant therapy for symptomatic individuals
- More specific therapy when available.

We need to provide genetic counseling for families. We need to provide a prophylactic therapy for those that are at high risk. We need to give a more appropriate anticoagulant for those individuals who’re undergoing therapeutic conditions that are at high risk.

And sometimes, we have more specific therapy that may be available, and we may need to treat the patient with. Some of the caveats for testing. We need to know the history and the status of the patient. Where is he in relation to his thrombotic event? Is he pregnant, is she pregnant? On birth control pills? Hormonal replacement therapy? Diabetic?
How to Test for Thrombophilia

**General Caveats**

- Know the history and status of the patient.
- Draw the appropriate samples: Non-traumatic draw and proper anticoagulants Properly processed and stored
- Use appropriate assays: Activity assays: AT, PC, FVIII, LA and APC-R Antigen assays: Free PS Genetic tests: Prothrombin, Factor V Leiden Other: Homocysteine
- Know assay limitations

Those things we need to know about the patients. We need to draw the appropriate samples, and we need to process them properly. We need to use the appropriate assays. We need to use activity assays when appropriate, antigen assays when appropriate, and genetic tests when appropriate.

Cautions that we need to take into account. We need to understand that there is no global test for thrombophilia. We have to perform a panel of assays, and that’s very important as to what to pick.

Thrombophilia Evaluation

**Cautions**

- Must perform panel of assays
- No global test
- Drugs and physiologic conditions influence results:
  - Oral anticoagulant therapy decreases PC and PS
  - Heparin can influence AT and PS levels
  - Pregnancy, BCP and HRT decreases PS and AT levels
- Levels decrease in pathologic conditions:
  - Acute phase of thrombosis and DIC
  - Severe liver disease
  - Post operative state

We need to know whether there’s physiologic conditions, pregnancy, or drugs, anticoagulant therapy or heparin, that we need to take into account when we evaluate the patient. I’m repeating these because they’re very important. And levels that are decreased in pathologic conditions need to be taken into account. Liver disease.

The acute phase immediately after a thrombotic event, and post surgery. Who should be evaluated? Well, mostly patients between the ages of 13 and 50 have a high probability of being thrombophilic if they have venous thrombosis. If you’re 60 years of age or greater, that risk is down.
And it may not be the case, especially if there’s some underlying condition such as a hip replacement or something like that. Thrombosis at unusual sites should trigger a thrombophilia workup. Causes of an unknown or spontaneous thrombotic event should trigger a thrombophilia workup. And if there’s a family history or a previous personal history of thrombosis, we need to work those patients up.

This slide shows, just describing what happens in protein C and protein S deficiencies if you’re homozygote. The average age of onset in protein C and protein S in homozygote, you’ll have, you’ll see the thrombosis at birth. In APC resistance, 28 years of age.

Heterozygotes, on the other hand, have a different time span, and for protein C and protein S, and also for antithrombin, it’s usually the average age is in the 20s. The APC resistance and the prothrombin mutation, the first thrombotic event with heterozygotes for APC resistance or factor V Leiden and prothrombin is in their 60s.

And that’s a very important point when we start talking about stratification. When should we workup a patient? The best time to workup a patient is when they’re asymptomatic and not on therapy. All other times, we can only give a tentative diagnosis.
We should not be putting a label on the patient when they are immediately post thrombosis or on heparin or Coumadin. Genetic tests can be performed any time. How do we go about approaching the thrombophilia workup? Our overall approach is as follows. We rule out all other causes.

**Title: When Should a Patient be Worked Up?**

<table>
<thead>
<tr>
<th>Optimum Time:</th>
<th>Asymptomatic and No Therapy</th>
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<tbody>
<tr>
<td>All Other Times:</td>
<td>TENTATIVE DIAGNOSIS ONLY</td>
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<tr>
<td>Symptomatic and Prior to Therapy</td>
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<tr>
<td>&gt;80%</td>
<td>Probably Normal</td>
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<tr>
<td>&lt;30%</td>
<td>Probably Abnormal</td>
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<tr>
<td>30-80%</td>
<td>Indeterminate</td>
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<tr>
<td>Any Time:</td>
<td>Genetic Assays</td>
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Myeloproliferative disorders, cancer, other things. Then we perform an aPTT assay. If it’s prolonged, we screen for heparin. That’s the most common cause of a prolonged PTT. If it’s still prolonged after screening for heparin, then we rule out a lupus anticoagulant and antiphospholipid syndrome.

We also do an anticardiolipin, an antiphospholipid antibody test, and a beta-2 glycoprotein-1 antibody test. If they’re positive, we repeat them in four to six weeks, and they may be transient or they may be permanent. And then, we perform a thrombophilia panel.

And then, that’s followed up with any reflexive tests or additional tests as warranted. Our recommended panel. We stratify on the basis of age. We use 60 years. If the patient is less than 60 years of age, we perform an APC resistance assay without factor V-deficient plasma.
If it’s abnormal, then we do a factor V Leiden DNA analysis. We do a protein C activity, and we do a free protein S antigen, factor VIII, antithrombin, and a genetic test for prothrombin 20210, and a plasma homocysteine.

If the patient is greater than 60 years when he has his first thrombotic event, we do an APC resistance assay, again without factor V deficient plasma. If it’s abnormal, we do the DNA test for factor V Leiden. We do a factor VIII, a prothrombin 20210, and a plasma homocysteine. You’ll notice that we do not do an antithrombin, protein C or protein S.

We’ve found that if you’re going to have a thrombotic event with protein C, protein S, or antithrombin deficiency, you’re going to have it before the age of 60. So this is a cost-effective strategy in stratification. If a patient has a thrombophilia, either diagnosed or undiagnosed, the patient needs to make lifestyle changes.
Patients with defects must make these changes. They must modify their behavior. They must eat better so they can reduce their homocysteine levels and just have a healthier lifestyle, quit smoking, exercise more. They need to make decisions about whether to use the birth control pill or hormonal replacement therapy.

We need to educate the patient for signs and symptoms and the potentials for treatment if you have a deficiency. And major complications such as surgery, we may need to treat the patient during that complication. Now in summary, I’d like to just say that thrombophilia is a multifactorial disorder.

It has risk factors that are genetic, acquired or physiologic. There’s probably 20 to 30 genes, maybe even more, that contribute to the risk for venous thrombosis. The genetic factors vary in their potential; some great, some less. And there’s a cooperativeness that increases their, greater than sum of their parts.

We need to evaluate patients based on this model. We need to evaluate who, when, and what criteria that we talked about in this discussion, and we need to do it in a cost-effective manner. And with that, the future holds for us a potential for increased testing but at reduced cost. And with that, I’d like to thank everybody for listening to this CD today.