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WHAT IS IN A NAME, OR A ROSE BY ANY OTHER NAME WOULD SMELL AS SWEET!!

Much of the confusion related to the field of Coagulation testing, arises from the names of the tests. In an effort to simplify the confusion I will describe the most troublesome nomenclatures that are related to the highest frequency of errors. This article will cover tests and nomenclature used by **Coagulation Consultants lab**, although it may be common or slightly different in other labs. The main tests are listed in brief and detailed subsequently.

A. **Heparin Induced Platelet Aggregation (HIPA), Heparin Induced Thrombocytopenia (HIT)/PF4/Elisa.** This is two separate tests for the same clinical condition which is thrombocytopenia and possible thrombosis associated with heparin administration. The HIPA test is a functional test which uses donor platelets and the patient's plasma to detect platelet aggregation in the presence of heparin. The HIT assay is an immunologic assay which is designed to detect the IgG antibody which binds to heparin and platelet factor 4. The HIT assay is very sensitive while the HIPA is more specific and these tests are described in detail below.

B. **Lupus-like anticoagulants, Tissue Thromboplastin Inhibition (TTI), Dilute Russel Viper Venom Time (DRVVT) with or without confirmation.** These are two test to screen for Anti-Phospholipid antibodies which are associated with thrombotic disorders and the details of these tests and why two are recommended is described below.

C. **Platelet antibody, direct or indirect and ADAMTS-13**. These are three different test that are descriptive of anti-platelet antibodies or in the case of ADAMTS-13 that detects the level of an enzyme needed to prevent Thrombotic Thrombocytopenia Purpura.(TTP). The Platelet Antibody direct method is the most sensitive and specific assay but requires 30 ml of whole blood, while the screen is less sensitive and less specific requires only 1-2 ml of serum. More details are listed below.

D. **Factor inhibitor (Bethesda titer), factor inhibitor screen (Circulating Anticoagulant**. The two assays here are distinguished by their names in parenthesis and amount to two very different methodologies. A factor inhibitor or Bethesda Titer is a very specific request for single factor inhibitor (e.g. most commonly Factor VIII) and measures the level of the antibody in the plasma that produces a factor deficiency of <25%. If the specific factor is unknown, one generally requests a Circulating Anticoagulant assay which will detect all types of inhibitors (e.g. Lupus-like anticoagulants or other factor inhibitors). These methods are described below.

E. **Factor V or Factor V Leiden**. This is a communication problem, usually between the person placing the order and the one taking the order in the lab. These are two very different tests, the Factor V is a simple coagulation factor assay for understanding why a patient is bleeding or seeing a patient's liver function, while a Factor V Leiden is a test to describe a mutation on the Factor V gene that is associated with clotting abnormalities. These are described in more detail below.

F. **Factor X or Anti-Factor Xa.** Here again is a common communication error for two very different tests. The Factor X is just a coagulation factor assay while the Anti-Xa is a monitor of heparin in plasma. The latter requires knowing what type of heparin is being given to the patient and since some of the new low molecular weight heparins have their own specific names (e.g. Lovenox, Enoxaparin, Fragmin, or Fondaparineux) the nursing staff may not recognize these as forms of heparin when ordering Anti-Xa. More detail is listed below.

A. HEPARIN INDUCED PLATELET AGGREGATION (HIPA) VERSUS HEPARIN INDUCED THROMBOCYTOPENIA (HIT)/PF4-ELISA

Heparin is a high molecular weight molecule that can under certain circumstances cause antibodies to be formed. This usually occurs after 3-5 days of therapy or within a day or two, if the patient has been exposed previously. Many antibodies are formed (IgG, IgM and IgA) but the antibodies that are associated with the HIT syndrome are usually IgG. These antibodies have specificity for both the heparin molecule and the platelet, more specifically, the platelet heparin binding glycoprotein called Platelet Factor 4 (PF4). Consequently, the antibody has the potential to cause thrombocytopenia and platelet plugging or thrombosis. This is clinically called Heparin Induced Thrombocytopenia and Thrombosis (HITT). Unfortunately, there are many tests described to determine the presence of a heparin antibody that may or may not be associated with either thrombosis or thrombocytopenia. In addition, the clinical diagnosis of HITT is made more difficult due to a thrombocytopenia associated with the initial administration of heparin that is not caused by antibodies since it is reversible and is not dependent upon the continued use of heparin.

The earliest test used to determine heparin antibodies (which we do not offer) is the Serotonin release assay and involves radioactive isotopic identification of the release of serotonin from normal platelets when exposed to heparin and a plasma sample with heparin antibody. This test although very difficult to standardize, was classified as the golden standard. An equivalently sensitive test is the HIT test (PF4- Heparin Elisa assay). The sensitivity of the HIT assay exceeds its specificity, meaning the test will be positive although many cases will never develop any clinical consequences of HITT. Therefore the HIPA (Heparin Induced Platelet Aggregation) test is used which is not as sensitive as the HIT but is more specifically associated with clinical thrombocytopenia and thrombosis. Unfortunately, the overall specificity is usually only 70%. So the clinician is faced with a dilemma of a test with good sensitivity and poor specificity and a test with fair specificity and poor sensitivity. With this background, which test should be ordered if any?

The most logical would be to order the HIT/PF4 Elisa and if positive, reflexively perform the HIPA to anticipate a clinical problem. Clinically, the thrombocytopenia should be obvious and the risk of thrombosis may be anticipated if the result of the HIT titer is noted as High Risk and confirmed with excess heparin along with a similarly positive HIPA. Both tests can produce positive, equivocal or borderline results, which may not present a high risk for the development of HITT. Regardless of all test results presently available, it is still a clinical decision to remove the patient from any form of heparin while looking for any signs of thrombosis. Thus many clinicians prefer to order only the HIPA, since they rely on clinical findings and realize the existence of the antibody may not be clinically relevant enough to remove the patient from all forms of heparin. To confuse the whole scenario even more, dialysis patients who have been diagnosed with HIT with positive antibody tests have been re-administered heparin after the heparin antibody titer normalized and have had no recurrence of either antibody or thrombosis, which has not been explained.

If the ordering physician is not explicit in the order for HIT or HIPA when he/she asks for heparin antibody assays, I would recommend calling them and explain the ambiguity or have them call us.

B. LUPUS-LIKE ANTICOAGULANTS/ ANTI-PHOSPHOLIPID ANTIBODIES

The term Lupus-Like Anticoagulant (LLA) is commonly used while being incorrect in two aspects. Although it was first described in cases of Lupus Erythematosis, it is not always found in these cases and secondly it is not an anticoagulant "in-vivo" but rather a thrombotic marker. What is characteristic of the LLA is that they are antibodies against phospholipid-protein complexes and as such they have the ability to prolong the "in vitro" APTT assays depending on the phospholipids used in the reagent. This is why they are referred to as anticoagulants. The antibodies are heterogeneous and can be detected in many assays that utilize small amounts of phospholipids. Reagents that have higher concentrations of phospholipids will not usually detect the antibody (e.g. The Prothrombin Time). Some of the proteins that are antigenic targets for these Anti-phospholipid antibodies and may explain their hypercoagulable associations are beta2GPI, Annexin V, Complement Factor H, high and low Mol. Wt. Kininogen, Prothrombin, Protein C, Protein S, Factor IX, C4b-BP and Thrombin modified ATIII. Some recent publications have shown a strong association of the antibody titer and the subsequent decreased levels of Beta2 Glycoprotein I and the fact that the latter protects the high molecular weight vovWillebrand factor from activating platelets (ergo thrombocytopenia and thrombosis associated with Lupus-like anticoagulants).

Many tests have been found which claim identification of these antibodies, to name a few DRVVT, dilute TTI, dilute PT, KCT, Texatarin Time and their associated normalization procedures with excess phospholipids (e.g. platelet neutralization or hexagonal phase phospholipids). However, there is a poor intra-correlation between all tests, so that some test will reflect positive results while other tests will be negative on the same samples. Historically, you will find positive correlations between pairs of tests, e.g. Anti-Cardiolipin, Anti-phosphatidyl-serine, Anti-Prothrombin and Antibeta2- glycoprotein I antibodies, but there are poor correlations between them all and between the clinical findings of thrombophilia. Therefore, the International Society of Thrombosis and Hemostasis has recommended the following steps (or algorithm) for the identification of a Lupus-like Anticoagulant.

I. A prolongation of a LLA sensitive APTT and in some cases PT also.
II. A prolongation of a circulating anticoagulant tests beyond 80:20 and 50:50 mixtures of Patient plasma: Normal plasma. (Note-normal plasma is only prepared from platelet free samples with normal APTT's).
III. Two positive screening test (e.g. DRVVT and TTI) with normalization in the presence of excess phospholipids.
IV. The first three tests remaining positive after 90 days. Plus , positive tests for Anti-Beta2 Glycoprotein I or Anti-prothrombin antibodies are more specific assays for the diagnosis of Anti-Phospholipid Syndrome.

The last criterion is very important due to the physiologic nature of these antibodies. They are believed to be constitutive with the purpose of tagging and facilitating the removal of activated platelets from the circulation. Activated platelets have exposed phospholipids, which bind the antibody and are consequently targeted for removal by the Reticular Endothelial System. Therefore, a clinical thrombotic event can be expected to transiently generate these antibodies, and it is difficult to diagnose the relevance of their presence as a result of the clinical condition or the cause of the condition. However, the persistence of the antibodies and a good clinical history (e.g. spontaneous abortions) can help in the diagnosis.

C. ANTI-PLATELET ANTIBODY (DIRECT OR INDIRECT) AND ADAMTS-13

Platelet antibody assays can be requested for any number of clinical conditions, mostly related to thrombocytopenia. Many tests are described for these conditions, whether related to Drug induced, Idiopathic Thrombocytopenia Purpura (ITP), Thrombotic Thrombocytopenia Purpura (TTP) or Neonatal Alloimmune Thrombocytopenia. The usual screen for platelet antibodies involves a patient's sera tested against normal washed platelets from several donors, looking for aggregate formation on flow cytometers. This is an Indirect Platelet antibody assay, since it only uses a patient's sera. The Direct assay requires a lot of blood (which is critical in the thrombocytopenic patient) for the analysis of antibodies on their platelets (also performed with flow cytometry and immunologic chromogenic markers).

Very recently a definitive diagnosis for TTP has become available which does not involve anti-platelet antibodies but is related to a deficiency of an enzyme that breaks down the stored form of vonWillebrand Factor "in vivo" (ultra-high molecular weight von Willebrand Factor(UH-vWF)). The existence of the UH-vWF in the circulation causes platelet aggregation and thrombocytopenia. A clinical diagnostic criteria for this form of thrombocytopenia is that plasma transfusion supplies the enzyme and transiently reverses the thrombocytopenia. The test for this abnormality is ADAMTS-13 assay, which is referring to the metallo-protease enzyme that degrades the UHvWF. The assays will determine the level of the enzyme and if the deficiency involves an antibody against the enzyme (acquired deficiency) or a congenital deficiency. The continued existence of antibody against the enzyme can cause the recurrence of TTP.

D. FACTOR INHIBITOR VERSUS INHIBITOR SCREEN

When someone wants to screen for an inhibitor and dose not specifically request an inhibitor titer, they want a Circulating Anticoagulant assay. This can be performed using the Activated Partial Thromboplastin Times (APTT) or the Prothrombin Time (PT) or both depending on which tests are abnormal. It usually involves several dilutions of the patient's plasma with normal (platelet free) plasma (e.g. 80:20, 50:50, 20:80). These dilutions are tested before and after incubation for one hour to determine if the inhibitor is fast or slow acting and at what dilution it normalizes. If normalization occurs at a low dilution, this would suggest a factor deficiency, while higher dilutions are required for a Lupus-like anticoagulant or factor inhibitors (which are usually exacerbated with incubation).

However, when an antibody titer is requested, this refers to a Bethesda Titer which involves serial dilutions of a patient's plasma (having a factor level of <25%) with normal reference plasma, incubated for several hours and then assaying for factor in each dilution to determine where the factor levels is between 25% and 75%. The percentage of normalization is converted to Bethesda Units and is then corrected for the dilution and reported as Bethesda Units. For example; A patient's plasma Factor VIII returns to 50% activity when diluted to 1:8 with normal reference plasma, this would amount to 1 Bethesda Unit x 8 (dilution)= 8 Bethesda Units.

E. FACTOR V VERSUS FV-LEIDEN

This problem is usually a communications error where the physician requests a Factor V(five) Leiden and the order comes through as a Factor V(five) and a Leiden. It is easy to differentiate what is actually ordered by looking at other tests on the patient or the patient's clinical condition.. If a patient is bleeding, the doctor probably wants a Factor V. If the patient is clotting or has had a clot or the other tests ordered are for thrombophilic testing, the doctor wants a Factor V Leiden. Factor V is a primary coagulation factor made in the liver and is independent of Vitamin K. Therefore, its level is a good marker of liver function abnormalities that can result in bleeding. Factor V Leiden is the name of a genetic marker of a mutation on the Factor V molecule that interferes with the degradation of activated Factor V and thus is associated with excessive clotting activity. The thrombotic risk for individuals that are heterogeneous for this mutation is 8 times higher than an individual without the mutation, while a homozygous individual is at 80 fold higher risk than normal.

F. FACTOR X VERSUS ANTI-FACTOR XA

Factor X(ten) is a coagulation factor produced by the liver and requires Vitamin K for it final synthesis. It is often requested when a patient is experiencing a prolonged Prothrombin Time due to the administration of Coumadin (a drug that interferes with the absorption of Vitamin K). Like many coagulation enzymes, Factor Xa(FXa), is an activated serine protease that is inhibited by the administration of heparin. Ergo, the term Anti-Factor Xa , which refers to a test that measures the level of heparin in the patient's plasma that inhibits activated FX. Usually, heparin levels are measured indirectly by the prolongation of the APTT test, but recently, due to the generation of antibodies by unfractionated heparin, newer low molecular weight heparins have become available. These low molecular weight heparins (e.g. Lovenox, Fragmin or Fondaparineux) cannot be assayed with the APTT and thus the Anti-Factor Xa assay has been substituted. The results of the Anti-Factor Xa are in units or micrograms of heparin/ml of plasma and the assay requires standardization with the same heparin being administered to the patient. Therefore, when the test is requested we must know what brand of heparin the patient is receiving. This gets confusing, since the nursing units don't always associate the newer forms of low molecular weight heparin with heparin, and will often deny your request for the type of heparin, saying that the patient is not on heparin. Another problem is often encountered is that these newer forms of low molecular weight heparins are administered subcutaneously and there is a lag period of around 3-4 hours before maximum levels are reached in the circulation. Thus some requests for chronic users will be for a pre and post injection assay of Anti-Xa levels to determine if there are any problems with elimination of the drug between dosages. In most cases, if there is normal kidney function and relatively normal body weight the drug does not need to be monitored at all. It is also relatively safe compared to unfractionated heparin in that it is not as antigenic as unfractionated heparin. Normal levels of Anti-Xa are usually referenced as the therapeutic or prophylaxis levels achieved 3-4 hours after the SQ administration. Most importantly, the blood collection must be centrifuged to eliminate platelets from the plasma otherwise they will normalize the heparin in the sample.