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Interpretation and clinical application of thrombophilia testing

1. Acquired Thrombophilia

- a. **Extractable nuclear antibodies (ENA)**
 - i. SM antibody titer
 - ii. RNP antibody titer
- b. **Cardiolipin antibody**
 - i. IgG antibody
 - ii. IgM antibody
- c. **Phosphatidylserine antibody**
 - i. IgG antibody
 - ii. IgM antibody
- d. **B2-Glycoprotein (IgG, IgA, IgM)** {above upper normal range for lab values; should be treated with Heparin/Lovenox}. B2-Glycoprotein may potentiate the effects (expression) of anti-cardiolipin antibodies; in such patients, aggressive treatment with Lovenox/Heparin is essential for a good pregnancy outcome. Such antibodies are part of the anti-phospholipid syndrome and are associated with infertility, IVF failure and early pregnancy loss as well as RPL. **{Am J Reprod Immunol 2006 Nov-Dec;56(5-6):337-44}, {Fertility Sterility 1998 Nov;70(5):938-44}, {Hamostaseologie 2005 Nov;25(4):391-3 }**
- e. **Flow Cytometry analysis (Natural Killer Cells)**{above upper normal range for lab values; should be treated with Heparin/Lovenox} and possibly steroids, depending on the quality of placental development.
- f. **Anexin V antibodies: [The presence of such antibodies is abnormal and thrombogenic]**

Anexin V is a natural anticoagulant that is expressed on the surface of the trophoblast. Such antibodies are part of the antiphospholipid syndrome. Their presence interferes with the role of membrane phospholipids and trophoblastic propagation. In addition, these antibodies promote fibrin deposition in the intervillous space. Increased fibrin deposition in the IVS is associated with poor pregnancy outcomes. Increased anti-annexin V antibodies are associated with recurrent pregnancy loss, infertility, IVF failure and early pregnancy loss. **{Am J Reprod Immunol 2006 Nov-Dec;56(5-6):337-44}, {Fertility and Sterility 2001;76(4):694-99}, {Hamostaseologie 2005 Nov;25(4):391-3 }**
- g. **Anti-Prothrombin antibodies: [The presence of such antibodies is abnormal and thrombogenic]** Such antibodies are part of the antiphospholipid syndrome. These antibodies are associated with increased thrombogenic activity in patients with lupus anticoagulant and antiphospholipid syndrome. Anti-prothrombin antibodies inhibit the inactivation of thrombin by antithrombin; this is thrombogenic. Patients with such antibodies and lupus anticoagulant are at increased risk for placental thrombosis. **{J Thromb Haemost 2005;3:1385-91}{Haematologica. 2006 May;91(5):699-702. Epub 2006 Apr 19, Br J Haematol. 2006 Oct;135(2):214-9. Epub 2006 Sep 11.}** In addition, the presence of such antibodies increases

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the risk for adverse perinatal outcome and are more likely to be present in patients with infertility (OR, 5.15 [CI, 2.1-12.7]) and recurrent pregnancy loss (OR, 5.4 [CI, 2.4-12.5]). *{Am J Reprod Immunol 2006 Nov-Dec;56(5-6):337-44}*

- h. Anti-platelet glycoprotein antibodies:** The presence of anti-platelet glycoprotein antibodies may be associated with platelet activation and increased thrombotic activity. *{Lupus 2005; 15:507-514}*

The presence of any of the above antibodies may interfere with the trophoblastic propagation and with the hemostatic mechanism causing poor placental development in addition to their effect on subplacental and intervillous thrombosis. Therefore any of the above should be treated with Aspirin 81 mg qd and in the presence of placental thrombosis with Lovenox/Heparin in addition to Aspirin.

2. Familial (genetic) Thrombophilia

- a. Protein S (functional) [If abnormally low should be treated with Lovenox/Heparin]**
Pregnancy may suppress protein S activity to as low as 40%. Values less than 40% should be treated immediately with Heparin/Lovenox. Values 40-60% are treated with aspirin only as long as the placenta has no thrombotic lesions. In the presence of placental thrombosis Heparin/Lovenox should be added. Values between 40 and 60 may be treated with aspirin only unless placental thrombosis is noted on the ultrasound. **Higher than normal values are of unknown significant and should not be considered abnormal.**
- b. Protein C (functional)** Protein C is not affected by pregnancy. {if abnormally low should be treated with Heparin/Lovenox regardless of placental appearance}. **Higher than normal values are insignificant and should not be considered abnormal.**
- c. Anti-thrombin III (functional)** Anti-thrombin III is not affected by pregnancy; if abnormally low should be treated with Heparin/Lovenox regardless of placental appearance due to increased risk for maternal DVT. **Higher than normal values are insignificant and should not be considered abnormal.**
- d. Homocysteine (serum)** {anything greater than the lab's normal reference is abnormal and should be treated with Metanx one tablet per day or if Metanx is not available, Folic acid, 2mg qd in addition to the multivitamins. In the presence of placental thrombosis however, Heparin/Lovenox should be added to the treatment}. A recently introduced prescription supplement contains the active form of folic acid that the body can use along with vitamin B12 and vitamin B6. The name of the supplement is Metanx and is indicated on all patients with all homozygous MTHFR mutations as well as the heterozygous with the C→T and compound (C→T and A→T) mutations. Folic acid is essential for the production of healthy DNA and RNA. Improper folic acid metabolism (like in patients with MTHFR gene mutations) leads to DNA damage and defective development. In addition, this may be responsible for increased risk for chromosomal defects.
- e. MHTFR Gene Polymorphism** {Positive is abnormal; homozygous should be treated with Folic Acid and Heparin/Lovenox and heterozygous with folic acid and aspirin only unless there are placental thrombotic lesions when Heparin/Lovenox should be added.} **See section (d) above.** The literature is controversial on its role for fetal loss but hyper-homocystinemia is a risk factor for pre-eclampsia. In our experience, the presence of MTHFR is most often associated with other thrombogenic factors and acts in concordance. We treat isolated MTHFR gene mutation with supplemental folic acid and aspirin. We monitor the placenta and if we note placental thrombosis then we add Lovenox/Heparin to the treatment *{Obstet Gynecol 2000; 96:45-9}*.

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- f. **Factor V Leiden Mutation {positive is abnormal and should be treated with Heparin/Lovenox}**. Factor V Leiden thrombophilia is the most common inherited form of thrombophilia. Heterozygosity for factor V Leiden occurs in up to 10% of the general US and European populations with a range of 2-20% depending on the population under study. The overall incidence of heterozygosity in Caucasians is one in 10 but homozygosity is one in 5000 or less; this is a lot less than the expected one in 100 according to Mendelian genetics. The reason for this discrepancy we believe is the fact that very few homozygous embryos survive the intrauterine life due to massive placental thrombosis. The prevalence varies considerably in different populations. The highest heterozygosity rate is found in Europe; the mutation is extremely rare in Asian, African, and indigenous Australian populations [Rees et al 1995]. The frequency of heterozygotes varies within Europe, with a prevalence of 10-15% in southern Sweden and Greece and 2-3% in Italy and Spain. In the US, heterozygosity for factor V Leiden was found in 5.2% of Caucasian Americans, 2.2% of Hispanic Americans, 1.2% of African Americans, 0.45% of Asian Americans, and 1.25% of Native Americans, reflecting the world distribution of the mutation {Ridker, Miletic et al 1997}. The prevalence also depends on the particular population sampled, with the factor V Leiden mutation present in approximately 15-20% of individuals with a first DVT, and up to 50% of individuals with recurrent venous thromboembolism or an estrogen-related thrombosis. During the pregnancy and puerperium there is an increased risk for adverse pregnancy outcomes {Human Reproduction 2002; Vol. 17(2):442-445}. There is an 8-fold increase in venous thromboembolism in non pregnant individuals and 40-fold in pregnant women. These risks are much higher in cases with additional thrombogenic factors. Homozygous is worse than heterozygous but both are abnormal. {<http://www.geneclinics.org/profiles/factor-v-leiden/details.html>}. Factor V causes low APC levels. Low APC levels may also happen due to other mutations as well as due to pregnancy and are associated with poor pregnancy outcomes {Thrombosis Haemostasis 2001;85:30-35}.
- g. **Factor II gene mutation {positive is abnormal and should be treated with Heparin/Lovenox}** In addition, women with this factor are at increased risk for maternal DVT and should use Lovenox prophylaxis during pregnancy and the puerperium regardless of the pregnancy status. The incidence of factor II polymorphism is 2-4% in heterozygous form and the incidence of homozygous is almost zero. The reason is that if a fetus is homozygous the risk for severe placental thrombosis and fetal loss is so high that only a very small number of such fetuses survive to be born alive. In the last 10 years in my practice I have not seen yet a homozygous factor II patient. Homozygous status is much worse than heterozygous but both are abnormal enough to require anti-thrombotic management. The presence of Factor II along with 4G/5G mutation increases the risk of thromboembolism {Thrombosis Haemostasis 2003; 90:1061-64}.
- h. **ACE insertion/deletion gene polymorphism (This is a very thrombogenic factor): DD homozygous is abnormal:** This is the gene responsible for the production of angiotensin converting enzyme (ACE). Insertion deletion defects in intron 16 are responsible for changes that lead to variable ACE activity. During pregnancy, marked changes in hemostasis take place and ACE D/D (Homozygous) genotype has been proposed as a new thrombophilic factor influencing pregnancy negative events. In addition to the well-known vasomotor functions, renin-angiotensin system is also involved in key events of the inflammatory process by increasing vascular permeability and contributing to the recruitment of inflammatory cells. Regarding hemostasis, several reactions are modulated by the renin-angiotensin system, and

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evidence exists for an association between the ACE D/D genotype and increased risk of thrombotic events. Moreover, ACE by bradykinin degradation reduces nitric oxide levels, therefore contributing to endothelial dysfunction. The renin–angiotensin system (RAS) has been shown to be involved from early stages of fetal/placental development. Expression of angiotensinogen, renin, angiotensin converting enzyme and angiotensinogen II type 1 receptor (AT1) has been demonstrated in first trimester deciduas and in and around the spiral arteries, which may influence vascular remodeling of the spiral arteries. Failure of physiologic transformation of the spiral arteries has been demonstrated in women undergoing preterm labor with or without intact membranes. ACE activity has been shown to be higher in preterm infants. ACE as a component of the RAS converts angiotensin I to angiotensin II which acts as a potent vasoconstrictor and also as a growth factor resulting in medial hyperplasia of the blood vessels. The DD genotype with its higher ACE activity has been shown to be a risk factor in pregnancies complicated by preeclampsia, intrauterine growth restriction and recurrent pregnancy loss, all of which have a common factor of defective placentation. Hence it is plausible to suggest that ACE/RAS abnormalities, manifesting as abnormal vascularisation of the placenta may be partly responsible for precipitating preterm labor among other placenta related complication. The combined presence of ACE polymorphism along with 4G/5G polymorphism is a potent thrombotic event leading to severe placental damage. *{J. Obstet. Gynaecol. Res. Vol. 34, No. 3: 301–306, June 2008, Braz J Med Biol Res 40(4) 2007, European Journal of Obstetrics & Gynecology and Reproductive Biology, 2008}*

- i. **APC Resistance: [Values <2.5 are abnormal and thrombogenic].** In addition, women with this factor are at increased risk for maternal DVT and should use Lovenox prophylaxis during pregnancy and the purpureum regardless of the pregnancy status *{Thrombosis Haemostasis 2001;85:30-35}*.
- j. **Plasminogen Activator Inhibitor type 1 (PAI-1) alone or in combination with 4G/5G gene polymorphism: [High values are abnormal and thrombogenic and the presence of one or two 4G alleles is abnormal]** This factor not only promotes increased risk for thrombosis (impaired fibrinolysis), but it has been found to directly inhibit trophoblastic invasion which may in turn lead to pregnancy failure. {Values above the high normal reference range are abnormal; treatment with Heparin/Lovenox should be considered if there are other abnormal factors or placental thrombosis. In patients with known PCOS Metformin 500 mg TID may be appropriate prior to and during the pregnancy up to 12 weeks. The presence of elevated PAI-1 along with Factor II polymorphism increases the risk for thromboembolism significantly *{Thrombosis Haemostasis 2003; 90:1061-64}*. PAI-1 expression in the decidual cells regulates the invasiveness of fetal trophoblast and increased levels of it lead to reduced invasion while low levels lead to molar pregnancy, placenta accreta and ectopic pregnancies *{Placenta 2000;21:754-62}*. Patients with increased PAI-1 levels are more likely to develop preeclampsia and poor perinatal outcomes *{Metabolism 2000;49(7):845-52, Thrombosis Haemostasis 1998;79:500-8}* as well as recurrent pregnancy loss *{Human Reproduction 2003;18(11):2473-77}*. The combination of this gene along with ACE polymorphism has detrimental effects on placenta quality with all associated complications.
- k. **Tissue Plasminogen activator (t-PA) deficiency:** Decreased tissue plasminogen is thrombogenic. t-PA catalyzes the production of plasmin from Lys-plasminogen. Plasmin in turn acts as a fibrinolytic by breaking down fibrin into fibrin degradation products. Therefore, t-PA deficiency leads to decreased plasmin production and hypofibrinolysis. Plasmin is inhibited by a-2 plasmin inhibitor (anti-plasmin) and thus fibrinolysis is inhibited. Free plasma plasmin is

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much more vulnerable to inhibition by a-2 plasmin inhibitor while plasmin bound to fibrin is less vulnerable. Plasmin inhibition when bound to fibrin is slow, well controlled and leads to physiological fibrinolysis. *{Women's Issues in Thrombosis and Hemostasis; Chapter 1:1-20 Martin Dunitz, London 2002 Brenner, Marder, and Conard, Editors}*.

- i. Alpha-2-antiplasmin (a-2 plasmin inhibitor):** *[High values are abnormal and thrombogenic]* Alpha2-Antiplasmin (a2-AP) is a serpin (serine protease inhibitor) and **is the main physiologic inhibitor of the fibrinolytic plasmin** in mammalian plasma. It is synthesized in the liver and is present in plasma at a concentration of about 1.0 nmol/mL. **Alpha2-antiplasmin inhibits fibrinolysis/clot degradation and is thrombogenic.** Human and murine a2-AP with molecular weight of 65 to 70 kDalt rapidly inactivate plasmin, resulting in the formation of a stable inactive complex, plasmin–a-2-AP. Plasmin is fibrinolytic and plasmin inactivation has prothrombotic properties. Lack of a2-AP improves the vascular patency after endothelial injury which is mainly because of the enhancement of endothelial cell healing via an over-release of vascular endothelial growth factor (VEGF) as a result of the exaggerated activity of plasmin no longer tempered by a2-AP. Moreover, the increased fibrinolytic potential in addition would reduce thrombotic vessel reocclusion. This dual effect might regulate the neointimal thickening after endothelial injury. Apart from the removal of fibrin, the fibrinolytic system also plays a pivotal role in **phenomena such as embryogenesis, ovulation, intima formation, proliferation, migration, tumorigenesis, and metastasis.** It has been reported that the levels of plasmin–a-2-AP complex in plasma are elevated in acute stroke, myocardial infarction, unstable angina, and arterial fibrillation. *{J Thromb Haemost. 2003 Aug; (8):1734-9, Thromb Haemost. 2001 Aug;86(2):640-6, Blood. 2003;102:3621-3628, Curr Pharm Des. 2006;12(7):841-7}*
- m. TAFI (Thrombin Activatable Fibrinolysis Inhibitor):** *[Abnormally high values inhibit fibrinolysis and exert thrombogenic activity]* TAFI is activated by thrombin and inhibits fibrinolysis contributing to increased clot stability and hypercoagulability. TAFI has different effects on venous vs. arterial thrombosis. It is highly thrombogenic in venous embolism but protective of arterial thromboembolism. This is in part the result of TAFI's anti-inflammatory (inhibition of bradykinin, C5a and osteopontin) effects which are important for the mechanism of arterial thrombosis. Since factor XI is a TAFI activator, this explains why factor XI deficiency does not confer protection against acute myocardial infarction; this implies that factor XI deficient patients have impaired TAFI activation and miss TAFI's anti-inflammatory activity. *{Pathophysiol Haemost Thromb 2003/2004;33:375-381}*
- n. D-dimer:** D-dimer is a marker for ongoing thrombosis and fibrinolysis. It's presence in the absence of any known thrombophilia associated factors, should alert the clinician to the presence of ongoing thrombosis on the placenta (IVS thrombosis). Even in the absence of ultrasonically recognizable blood clots in the placenta there is strong probability that microscopic level clotting is taking place at the level of the intervillous space with fibrin deposition over the chorionic villi. Circulating D-dimer as well as other fibrin derivatives may possess a small but significant amount of active thrombin that could serve to propagate the coagulant process elsewhere in the circulation. *{J Lab Clin Med 1983; 102:220-30}*
- o. Factor XII :** Increased levels **increase the risk for intravascular thrombosis and clot propagation.** However, factor XII deficiency does not seem to be a risk factor for bleeding. The literature findings on this factor are still controversial and more research is needed to clarify factor XII functions under various circumstances. For example, Factor XII deficiency in the presence of prolonged APTT (lupus anticoagulant) is thrombogenic and associated with poor

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perinatal outcomes (***Ann Rheum Dis* published online 4 Oct 2006**). A new hypothesis is presented on the function of factor XII, which is postulated to be a "missing link" between acute stress and transient hypercoagulability. The implications of this idea are developed to show how chronic stress, which involves activation of hypertension and migraine as well as hypercoagulability, can cause cerebrovascular disease. "Acute stress" is defined as "the normal short-term physiological response to the perception of major threats or demands". "Chronic stress" is "the abnormal ongoing physiological response to the continuing perception of irresolvable major threats or demands". The factor XII hypothesis is as follows: Acute stress includes release of epinephrine by the adrenal medulla. Epinephrine activates platelets by binding to alpha-2 adrenergic receptors. Activated platelets convert pre-bound factor XII to its active form, which then initiates the intrinsic coagulation cascade. This can be called the "activated platelet initiation pathway" for coagulation. Neither tissue factor nor pre-formed thrombin is required. Thrombosis proceeds to completion, but only a minute amount of thrombin is formed, and the process normally stops at this point. In people who lapse into a state of chronic stress, essential hypertension, which is also a manifestation of stress, synergizes with hypercoagulability: there is both a baseline rise in blood pressure and systemic platelet activation as well as superimposed labile rises of both. Upregulation of these two stress parameters is atherogenic: epinephrine-activated platelets stimulating thrombin formation interact with endothelial cells activated by angiotensin II to cause, first, smooth muscle cell proliferation, which is a histological hallmark of atherosclerosis, and, lastly, a symptomatic thrombotic occlusion-the stroke. The migraine symptoms which often accompany this process are a marker of chronic stress and ongoing pathophysiologic damage. Therapeutic predictions are made regarding novel ways of blocking stress-induced hypercoagulability and hypertension. Hypercoagulability could be targeted by monoclonal antibodies directed against the platelet-specific alpha-2 adrenergic receptor or the (putative) platelet receptor for Factor XII; hypertension could be treated with monoclonal antibodies directed against the beta-adrenergic receptor in the juxtaglomerular apparatus or by surgical denervation of the kidneys, either of which would decrease the renin release which helps drive the hypertension. **{*Med Hypotheses. 2006;67(5):1065-71. Epub 2006 Jun 6*}**

- p. **Factor XI:** Is a cofactor for the activation of factor IX by means of intrinsic pathway. Factor XI binds to the platelet surface and gets activated (FXIa) and then along with FVIII activates FX to FXa. FXa then along with FV converts Prothrombin to thrombin. **Increased levels are thrombogenic.**
- q. **Factor IX:** Increased values are significant for **increased risk for thrombosis.**
- r. **Factor VIII:** Increased values are significant **for increased risk for thrombosis.** Increased Factor VIII levels are associated with increased risk for RPL (***Thrombosis Hemostasis, 2004; 91: 694-9***)
- s. **Factor XIII:** Increased values are significant for **increased risk for thrombosis** and are associated with increased risk for recurrent pregnancy loss. Factor XIII (FXIII) is a protransglutaminase that, after activation, cross-links fibrin chains and several plasma proteins, most importantly alpha (2) plasmin inhibitor, to fibrin. FXIII strengthens the fibrin clot by covalent bonds and protects fibrin from the prompt elimination by the fibrinolytic system. In the last two decades, FXIII has emerged as a key regulator of fibrinolysis. FXIII is also present in platelets, monocytes, and macrophages, but this cellular form does not contribute significantly to maintaining hemostasis. FXIII deficiency is a life-threatening bleeding diathesis whose clinical consequences are well studied. In contrast, the involvement of FXIII in thrombotic disorders and its association with the risk of such diseases are less clear. This

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review gives an account of the data accumulated mainly in the last decade on the association of FXIII with atherothrombotic diseases and presents conclusions and hypotheses drawn from these data as well as exposing the limitations of the published studies and our knowledge on this topic. The involvement of FXIII in atherogenesis, its role in coronary artery disease, atherothrombotic ischemic stroke, and peripheral artery disease are discussed, with particular reference to the association of FXIII levels and polymorphisms with the risk of these diseases

- t. **Fibrinogen:** Elevated levels may cause excessive clotting in the IVS and lead to placental necrosis and thrombosis.
- u. **Lipoprotein (a):** This is a highly thrombogenic protein. Lipoprotein (a) modulates fibrin-plasminogen interactions by inhibiting plasminogen binding to fibrin. **{*Semin Thromb Hemost 1982;8:2-10*}** Its presence has been associated with increased incidence of adverse pregnancy outcomes. **{*Acta Obstet Gynecol Scand 2006;85(10):1172-8, Atherosclerosis 2004 Sep;176(1):181-7*}**
- v. **Protein Z:** Protein Z is a vitamin K-dependent plasma protein that serves as a cofactor for the control of the coagulation factor Xa. **Protein Z deficiency is associated with an enhanced risk of severe placental insufficiency soon after the connection of maternal and fetal circulations.** **{*Blood 2002;99:2606-08*}** Protein Z deficiency was found to be more common in patients with pregnancy complications (pre-eclampsia, IUGR, and fetal demise, and recurrent unexplained embryo loss or fetal demise). **{*Am J Obstet Gynecol 2005;193:1698-702, Blood 2003;101:4850-52*}** Protein Z deficiency along with Factor V is severely thrombogenic and lethal in the presence of Homozygous Factor V polymorphism.
- w. **Sticky Platelet Syndrome: (Platelet Aggregation):** Approximately 22% of the patients with recurrent pregnancy loss (Habitual aborters) suffer from sticky platelet syndrome. Aspirin is the only treatment for this condition, and that's why aspirin takes care many of the mild forms of Thrombophilia related recurrent pregnancy loss. **{*Hematology/Oncology Clinics of North America 2000; October: 1117-31*}**
- x. **Interleukin 2(IL-2) and Interleukin 2 Receptor (IL-2R):** These are not thrombophilic factors. Only high values are abnormal and they mean that there is possibly some form of placental inflammation secondary to placental necrosis from thrombotic lesions. This test is only done in patients with strong suspicion of placental/decidual necrosis and protracted labor that does not respond well to Indomethacin/Procardia protocol. Such patients may also have elevated CRP levels and respond better to weekly 17-alpha-hydroxy-progesterone injections.
- y. **C-Reactive Protein (CRP):** This is not thrombophilia. CRP is a marker of placental apoptosis and chorionic villi degeneration and necrosis. We use it to document the degree of ongoing damage for treatment monitoring and also to confirm degeneration of the placenta when ultrasound indicates chorionic villi damage in the absence of any thrombophilic factors in the mother's blood. In addition CRP is a marker of inflammation. Inflammation has evolved into a very significant contributor to arterial thrombotic events (cardiac and cerebral) and its presence is associated with increased risk for such events. Recently, a gene mutation has been identified in individuals with increased levels of CRP. Individuals homozygous for the gene +1444C>T have higher circulating concentrations of CRP. **{*International Journal of Epidemiology 2006;35:922-931*}**. Pregnant patient with high CRP and preterm labor may benefit from the use of weekly 17-alpha-hydroxy-progesterone injections in addition to Indomethacin.