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EFFECTS OF ANTICOAGULANT THERAPY ON HYPERCOAGULABLE TESTING AND OTHER ISSUES AFFECTING RESULTS

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INTRODUCTION

Hypercoagulable testing is typically ordered for patients with a family history of thrombosis, a personal history of recurrent venous or arterial thrombosis, an unexplained thrombotic episode, or thrombosis in unusual locations. Lupus anticoagulant (LA) testing is frequently ordered when someone has had an unexplained thrombotic episode, has had recurrent miscarriages or late pregnancy complications, particularly in patients with an underlying autoimmune disorder, and/or as a follow-up to a prolonged PTT test.

Varied circumstances, including simply the use of anticoagulant therapy frequently interferes with the results and interpretation of hypercoagulable testing.

Patients admitted to the hospital with thrombosis often require immediate and prolonged anticoagulation, regardless of whether the thrombotic event was due to an underlying hypercoagulable disorder. This presents a diagnostic dilemma.

HEPARINS

Heparin anticoagulation therapy may cause false positive results for lupus anticoagulants, including prolonged aPTT and aPTT mixing studies. A thrombin time test may be done to rule out heparin contamination. Heparin has been shown to prolong the dilute Russell viper venom time (DRVVT) result, as well as partial thromboplastin time (PTT)-based LA test results. Therefore, anti-heparin agents are added to some commercial DRVVT kits to neutralize the effect of heparin in the specimen.

Results of other hypercoagulable testing may also be affected by anticoagulation therapy. Heparin works by potentiating antithrombin III activity; therefore, antithrombin III activity is frequently decreased in patients on heparin. However, antithrombin III can be checked in patients on Coumadin. Other situations may cause acquired antithrombin III deficiency including recent thrombosis, liver disease, consumptive coagulopathy such as DIC, nephrotic syndrome and heparin therapy. Congenital antithrombin III deficiency is rare and these patients present with thrombosis early in life that generally includes a significant family history of thrombosis. There is no clinically available genetic testing for antithrombin III deficiency.

WARFARINS

The oral anticoagulant warfarin has been reported to cause weakly false-positive DRVVT results, although LA testing may be feasible in the presence of warfarin.

Coumadin can affect other hypercoagulable testing. Physicians should remember that Coumadin therapy will cause not only a decrease in clotting factors II, VII, IX and X, but will decrease protein C and S levels. These tests should not be ordered in patients on Coumadin as the results would be low as expected. Protein C and S may also be low at the time of acute thrombosis, and in patients with liver disease. Further, protein S activity may be low in pregnant females, women on oral contraceptive pills, or hormone replacement therapy due to an increase in C4 binding protein.

INHIBITORS

Strong factor VIII inhibitors (specific antibodies against factor VIII) can decrease factor VIII levels causing false positive LA tests with prolongation of both aPTT and aPTT mixing studies. This is particularly important in elderly patients with underlying autoimmune disorders and bleeding symptoms.

If LA testing is ordered for evaluation of prolonged aPTT, in addition to testing for lupus anticoagulant, on occasion it is necessary to test for coagulation factor VIII levels.

Any causes for prolonged aPTT should be ruled out. Coagulation factor assays XII, XI, IX and VIII can be ordered to rule out factor deficiencies that may cause a prolonged PTT and bleeding episodes. Factor assays also help in detecting lupus anticoagulant as decreased levels of all factors, particularly those in the intrinsic pathway, have been reported in patients with lupus anticoagulant. This is a reagent dependent *in vitro* phenomenon.

Lupus anticoagulant may interfere with other hypercoagulable testing**.** For example, some patients with lupus anticoagulant have acquired activated protein C resistance in the absence of genetic mutations such as factor V Leiden, Cambridge or HR2. This was also reported to be associated with a trend toward increased thrombosis risk compared to those with normal APC-R, reflecting an *in vivo* pathophysiologic effect of lupus anticoagulant, rather than merely an *in vitro phenomenon*, even with the second generation APC-R assay.

Testing for lupus anticoagulant is sometimes ordered for evaluation of pregnancy complications. Physicians should be aware that lupus anticoagulant testing is frequently positive during pregnancy; however, if the patient has no clinical manifestations of an antiphospholipid antibody syndrome, treatment should *not* be initiated.

OTHER

Certain medications, other than anticoagulant drugs, cause positive lupus anticoagulant results. Antibiotics, particularly penicillins, are frequently associated with positive lupus anticoagulant results in the pediatric population; however, in this case there is no increased risk for thrombosis.

NEWER ANTICOAGULANTS

The direct thrombin inhibitors such as argatroban, lepirudin, bivalirudin and new oral direct thrombin inhibitors such as dabigatran, (that was approved by the FDA in 2010 for treatment of patients with nonvalvular atrial fibrillation) can cause significant interference with hypercoagulable testing.

Direct thrombin inhibitors are being increasingly used as alternative anticoagulants. These drugs present new challenges for the monitoring of appropriate anticoagulation. Studies have demonstrated that direct thrombin inhibitors can interfere with a number of coagulation-based assays, including testing for lupus anticoagulants using the DRVVT or PTT-based assays. They can also prolong PT, INR, aPTT, aPTT mixing studies, thrombin time, and can actually produce a false positive lupus anticoagulant result, including specific lupus anticoagulant testing such as hexagonal lipid neutralization testing and DRVVT. Anticardiolipin and beta-2 glycoprotein I antibodies can be falsely positive in patients on direct thrombin inhibitors. Clotting factor levels done by functional assays also appear to be very low, possibly due to the inhibitory effect of direct thrombin inhibitors. Most importantly, the activated protein C resistance ratio may actually demonstrate a false negative result which would miss the diagnosis of factor V Leiden mutation. Currently there is no laboratory method to reliably detect and neutralize the effect of direct thrombin inhibitors, therefore, hypercoagulable testing should not be ordered in patients on those agents.

SUMMARY

Many factors interfere with results and interpretation of hypercoagulable studies. When possible, hypercoagulable testing should be done outside of an acute event, including thrombosis, infection, inflammation, and most definitely prior to the initiation of anticoagulation therapy.

Factor V and prothrombin DNA testing is fairly definitive. Test results for protein S, C, and antithrombin III deficiencies are more difficult to interpret as environmental factors can influence the results. Clinical and family history should always be used to aid in testing interpretation. It is important to rule out acquired protein S, C, and antithrombin III deficiency prior to establishing a diagnosis.

Treatment with Coumadin can interfere with protein C and S testing. Ideally, Coumadin use should be held for two weeks prior to undergoing these tests. Alternatively, heparin use can replace the Coumadin during that time frame. Acquired protein S deficiency is quite common, frequently caused by factors such as pregnancy, liver disease, inflammatory conditions, and thrombo-embolism. Acquired antithrombin deficiency can result from mild liver disease, acute thrombosis, nephrotic syndrome, and heparin anticoagulant therapy. Protein S assays are *not* reliable during pregnancy and heparin therapy can result in a false positive antithrombin III test result; but, antithithrombin III can be checked on Coumadin.

It is always important to remember that protein C, S, and antithrombin III congenital deficiencies are rare and should be suspected in strongly thrombophilic patients: i.e., those with a venous thromboembolism prior to age 50, recurrent venous thromboembolism, or with an extensive family history of thrombosis. When results are borderline, repeat testing and comparative studies of other family members can be appropriate.

Finally, direct thrombin inhibitors cause significant interference with *all functional* coagulation assays and hypercoagulable testing should not be ordered in patients on these agents.

REFERENCES

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