Saint Luke's Regional Laboratories
Clinical Laboratory Letter

April 2010

Shigella Outbreak Locally

The Missouri Department of Health & Senior Services, along with the Kansas City Missouri Health Department recently issued a bulletin alerting physicians to an increased number of *Shigella sonnei* infections reported in the Kansas City Metro area. As of April 14, there had been 252 cases reported locally since the beginning of 2010.

Symptoms of shigellosis include diarrhea, fever, nausea, vomiting and cramps. Transmission occurs through direct contact with an infected person or contaminated food or water. Shigellosis is diagnosed by stool culture, which should be performed in all suspected cases prior to therapy. Susceptibility testing is routinely performed on all *Shigella* isolated by Saint Luke's Regional Laboratories' Microbiology. The Health Department recommends treatment with azithromycin or ciprofloxacin due to the frequent resistance of *Shigella* to ampicillin and trimethoprim/ sulfamethoxazole. Shigellosis is a reportable disease that should be communicated to the local health department within 24 hours of diagnosis.

Understanding Lupus Anticoagulant

Antiphospholipid antibodies are autoantibodies directed against phospholipid-protein complexes. These antibodies are associated with an increased risk for venous and arterial thrombosis as well as miscarriage. The two main types of antiphospholipid antibodies are lupus anticoagulant (LA) and anticardiolipin antibodies. Testing for LA should be limited to patients who have a significant probability of having the antiphospholipid syndrome or who have an unexplained prolonged aPTT in the course of routine coagulation testing. The antiphospholipid syndrome is diagnosed in patients with a history of venous thrombosis, arterial thrombosis or recurrent pregnancy loss in the presence of persistent antiphospholipid antibodies.

Lupus anticoagulants (LA) were first discovered in the 1950's in patients with systemic lupus erythematosis who had prolonged activated partial thromboplastin times (aPTT). Today we know that the name lupus anticoagulant is a misnomer because these autoantibodies are not restricted to patients with lupus and they rarely cause bleeding. LA may occur in the absence of any known underlying disease or be associated with autoimmune diseases, medications, infections, lymphoproliferative disorders, or malignancy.

Unfortunately, no single clotting test detects the many types of antibodies that constitute LA. To circumvent this problem, the International Society on Thrombosis and Hemostasis (ISTH) has devised a set of diagnostic criteria for LA:

- Prolongation of a phospholipid dependent clotting test designed to be sensitive to LA
- Demonstration of an inhibitor by showing incomplete correction of a 1:1 mix of patient and normal pooled plasma
- Demonstration of phospholipid dependence by showing shortening of the clotting time by addition of more phospholipid
- Ruling out the possibility of a coexisting specific factor inhibitor

LA prolong various phospholipid dependent clotting times because they bind to phospholipid-protein complexes, which are an essential component of the coagulation cascade.

The most commonly used screening test for LA is the activated partial thromboplastin time (aPTT). When the aPTT is prolonged to >40 seconds, circulating anticoagulants are distinguished from factor deficiencies by mixing one part of patient plasma and one part of normal plasma and then repeating the aPTT on the mixture. Results are read immediately and after one hour incubation at 37^{o} C. If the immediate result is >5 seconds of the normal plasma, the result is reported as no correction. If the immediate mix corrects within 5 seconds of the normal plasma, one hour incubation is performed. Incubated results that are 5 seconds or longer than the normal control are reported as no correction.

If the APTT does not correct, the hexagonal phase phospholipid test (HPPL) is performed as a

confirmatory test for LA. This test derives its name from the use of a soybean extract of phospholipid that retains its hexagonal structure and avidly binds LA. If LA is present in a patient's plasma, a shorter aPTT should be obtained after addition of this phospholipid. A difference of 8 seconds or greater between the tube containing phospholipid and the control tube is interpreted as positive. The combination of a prolonged aPTT that does not correct after a 1:1 mix and a positive HPPL is consistent with a lupus anticoagulant and no further testing is necessary. Direct thrombin inhibitors and Factor VIII inhibitors can cause a false positive reaction and should be ruled out.

If the hexagonal phase phospholipid test is negative, a dilute Russell viper venom time (dRVVT) can be run as an additional screening test. Russell viper venom activates factor X to initiate the common coagulation pathway. Patient plasma is tested with and without extra phospholipid. If LA is present, it binds to phospholipid, thereby prolonging the dRVVT clotting time. If the ratio of patient plasma to patient plasma plus extra phospholipid is 1.28 or greater, the result is reported as positive.

Occasionally, LA results meet some, but not all ISTH criteria for positive LA. When one confirmatory test is positive but another is negative (*e.g*. positive HPPL and negative DRVVT) a patient most likely has a LA because no single test is 100% sensitive. The positive test should be repeated 12 or more weeks later to determine if it is persistent.

Unlike LA, antiphospholipid antibodies are detected by enzyme immunoassays. APL include anti - cardiolipin and antiB2glycoprotein1 antibodies. They should be ordered at the same time as LA because results are discordant in ~30% of patients.

Discriminating between TTP-HUS and DIC in Adults

Disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS) are potentially life-threatening microangiopathic hemolytic anemias (MAHA) that have similar overlapping clinical presentations. TTP-HUS is classically described as being associated with the following pentad of findings, although the typical TTP-HUS patient rarely presents with all five of these features:

- Microangiopathic hemolytic anemia
- Thrombocytopenia
- Fever
- Acute renal insufficiency (elevated creatinine)
- Neurological symptoms

Because these signs may also be seen in an acutely ill patient with DIC, differentiating TTP-HUS from DIC may be difficult. However, this distinction is essential since early recognition of TTP-HUS and treatment with plasma exchange may be lifesaving.

In a recent retrospective case-controlled study, researchers analyzed CBC, prothrombin time (PT), partial thromboplastin time (PTT), D-dimer, fibrinogen and LDH results to determine if routine laboratory tests could discriminate between TTP-HUS and DIC (Amer J Clin Pathol 2010;133:460-465). A total of 27 adult patients diagnosed with TTP-HUS and 51 matched control cases of DIC were examined. Multivariate regression analysis showed that only a normal or slightly elevated PT and marked thrombocytopenia had a statistical association with TTP-HUS.

A platelet count less than 20 x 10^3/uL was 59% sensitive and 86% specific for TTP-HUS. A normal PT or PT less than 5 seconds above the upper limit of normal (ULN) was found in 93% of TTP-HUS patients compared to 43% of patients with DIC. As the table below shows, the combination of these two parameters increased specificity for TTP-HUS to 92%, however the sensitivity was only 52%.

Parameter	Sensitivity	Specificity
Platelet count < 20 x 10^3/uL	59	86
PT < 5 sec longer than ULN	93	57
PC < 20 x 10^3/uL & PT < 5 sec longer than ULN	52	92

Despite a tendency for higher D-dimer levels in DIC cases, this parameter as well as LDH, hemoglobin and fibrinogen results failed to distinguish TTP-HUS from DIC.

These findings may be helpful in evaluating MAHA cases that are suspicious for TTP-HUS. The authors recommended that emergent plasma exchange should be initiated in MAHA cases with marked thrombocytopenia and a normal or slightly elevated PT. They further suggest that a blood sample for ADAMTS13 activity be collected prior to plasma exchange to increase the diagnostic accuracy for TTP-HUS.