Saint Luke's Regional Laboratories
Clinical Laboratory Letter

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Quantiferon and T-spot for *Mycobacterium tuberculosis* Detection

Tuberculosis (TB) remains one of the most prevalent infectious diseases worldwide, with an estimated 9 million active infections annually, and 2 billion latent infections. Attributable mortality is approximately 2 million deaths globally per year. Overall, both active and latent TB infections have declined in the United States since 1998.

Tuberculin skin testing (TST) was a mainstay of latent and active TB infection prior to availability of blood assays for *Mycobacterium tuberculosis* (MTB) in 2001. Disadvantages of TST include the challenge of proper administration and interpretation, as well as false-positive results due to non-tuberculous mycobacteria infection and BCG administration.

TB blood assays are based on the principle of interferon-gamma being critical to regulation of cell-mediated immune response to MTB infection; hence their designation as interferon gamma release assays (IGRAs). Currently available FDA-approved IGRAs for MTB include Quantiferon and T-spot TB. Both assays measure the interferon-gamma response to specific MTB proteins, including early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10). Because these assays quantitate a biologic response, testing of a fresh blood specimen with viable white blood cells is crucial to obtaining accurate results.

The CDC has published guidelines for use of blood assays for the diagnosis of latent and active TB (MMWR 2010;59,No. RR-5).

- In general, an IGRA may be used in all situations for which TST is indicated. An IGRA is the test of choice in two instances; for people who have received BCG, either as a vaccine or as chemotherapy and under circumstances where the tested person is unlikely to return to have the TST read.
- Either TST or IGRA may be used to test contacts of people with active TB infection, and in screening for occupational exposures.
- Routine testing with both TST and an IGRA is not generally recommended. Exceptions include suspected active TB in immuno-compromised patients, or indeterminate results from either test.
- TST testing is preferred for children aged <5 years.

Neither IGRA nor TST can distinguish active from latent tuberculosis. CDC recommends that persons with a positive TST or IGRA be evaluated for the likelihood of TB infection. A diagnosis of latent TB requires that active TB be excluded by history & physical examination, chest X-ray, and cultures when indicated. Although both sensitivity and specificity of the IGRA tests is high, negative results are not sufficient to exclude infection in suspected active cases.

Saint Luke's Regional Laboratories offers IGRA testing through a reference laboratory, which has recently converted from Quantiferon to T-spot TB. Specimen requirement is one green-top sodium heparin tube of blood, which must be transported at ambient temperature as soon as possible, since testing must begin within 30 hours of specimen collection.

Dabigatran Monitoring

Dabigatran Etexilate (Pradaxa®) is an oral direct thrombin (factor IIa) inhibitor approved by the FDA to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Dabigatran etexilate is a prodrug that is rapidly absorbed from the GI tract and then converted to its active form, dabigatran. Dosing for patients with normal renal function is 150 mg by mouth twice daily. Therapeutic levels are reached within 30 minutes to two hours following oral administration. Steady state is reached within 2 to 3 days. The drug is excreted unchanged via the kidneys (~80%) with the remainder excreted via bile. Circulating half life is 12-17 hours.

Supra-therapeutic concentrations of dabigatran result in modest elevations of INR (~2.0). However the effect on INR is variable and unpredictable. INR should not be used as a measure of the anticoagulant effect of dabigatran.

There is a reasonable, non-linear correlation between dabigatran plasma concentration and the activated plasma thromboplastin time (aPTT). Dabigatran prolongs the aPTT to 1.5 – 2.5 times the control. At a dose of 150 mg bid, less than 10% of patients have aPTTs greater than 65 sec (or 2 times control) when measured 12 hours after dosing. An aPTT >2.5 x control may indicate over-anticoagulation.

Thrombin Time exhibits a linear correlation with plasma concentrations of dabigatran up to 400 ng/mL. Thrombin time may be elevated as much as 10 to 20 times control in patients with therapeutic plasma concentrations of dabigatran. Thrombin time may be too sensitive for routinely monitoring of dabigatran because in many instances an endpoint is never reached.

Dabigatran affects the activated clotting time (ACT) but no systematic investigation has been undertaken. Ecarin clotting time is a specific test that shows a close linear correlation with the plasma concentrations of dabigatran. However, this test is not generally available in hospital labs.

The Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial demonstrated lower bleeding and hemorrhagic stroke rates with dabigatran than with heparin. However, the incidence of dyspepsia and major GI bleeding were higher with dabigatran than warfarin. Bleeding risk is increased with concomitant use of antiplatelet medications.

Patients with therapeutic levels of dabigatran are at increased risk of bleeding during invasive procedures or surgery. Dabigatran should be discontinued at least 24 hours prior to elective surgery depending on the degree of renal impairment and risk of bleeding. In patients with normal renal function and a standard bleeding risk, discontinuation of dabigatran 24 hours before surgery will decrease plasma levels to approximately 12-25% of steady state trough levels. Dabigatran should probably be discontinued for 2-4 days prior to surgery for patients at higher risk of bleeding or for major surgery. For patients at

high risk of bleeding, a thrombin time can be performed 6-12 hours before surgery. An elevated thrombin time, in the absence of heparin or other direct thrombin inhibitors, is an indication of the continued presence of dabigatran.

Currently, no antidote is available to reverse the anticoagulant effect of dabigatran. The off label use of recombinant activated factor VII, nonactivated prothrombin complex concentrates, and activated prothrombin complex concentrates may reverse the anticoagulant effect but have not been adequately studied.

Pancreatic Cyst Fluid

The majority of pancreatic cysts can be classified into 4 main categories: pseudocyst, intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN), and serous cystadenomas. Risk of malignancy is low in pseudocysts and serous cystadenomas but higher in IPMN and MCN. The clinical dilemma is to distinguish high risk cysts that may require resection from lower risk cysts that may be followed.

Many pancreatic cysts can be classified solely on the basis of clinical and radiologic data, while a minority requires additional tests on cyst fluid. Cytology, CEA and amylase are most commonly ordered.

CEA is generally undetectable or low in aspirates from pseudocysts and serous cystadenomas. CEA is often helpful in separating mucinous cysts (IPMN and MCN) from nonmucinous cysts. IPMN and MCN produce fluid with increased CEA due to the mucinous lining of these cysts. In general, the higher the CEA value, the more likely the cyst is a mucinous cyst, with the associated risk of malignancy.

Amylase may be helpful in differentiating pseudocyst from serous cystadenoma. Cyst fluid amylase is most always substantially elevated in pseudocysts, with values often exceeding 1000 U/mL, but low in serous cystadenoma. IPMN have variable amylase levels since many communicate with the pancreatic ductal system.

Molecular testing for KRAS mutation may also be helpful in identifying mucinous cystic tumors. The combination of KRAS mutation with CEA elevation appears to be more sensitive for detection of malignancy than either test alone.