(F)utility of Leukocyte Alkaline Phosphatase

Historically, the diagnostic utility of leukocyte alkaline phosphatase (LAP) activity (measured as the LAP score) was in the differential diagnosis of leukocytosis (Leukocyte Alkaline Phosphatase in Hematology 4th ed. McGraw-Hill: New York 1990). The LAP score was usually low in chronic myeloid leukemia (CML), which helped distinguish CML from other myeloproliferative neoplasms with leukocytosis and inflammatory leukemoid reactions, both of which had a normal or increased LAP score. Additionally, it was noted that the LAP score was also low in patients with paroxysmal nocturnal hemoglobinuria (PNH), although this was not used as a diagnostic test for PNH. In the laboratory, the LAP score is determined using a cytochemical stain on a peripheral blood film followed by a semiquantitative visual estimation of the degree of staining in neutrophils. Thus, the test is inherently limited in its reproducibility and accuracy. Finally, even before the molecular pathogenesis of CML and PNH was fully understood, it was recognized that the LAP score may not always be low in CML and PNH, and can be low in some cases of other myeloproliferative neoplasms and myelodysplastic syndromes.

The current WHO classification of chronic myeloproliferative neoplasms does not use the LAP score at all (WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. IARC: Lyon 2008). CML is defined by the presence of the BCR/ABL-1 rearrangement, which underlies the pathogenesis of CML and is the basis of treatment of CML using tyrosine kinase inhibitors such as imatinib mesylate. If a myeloproliferative neoplasm is positive for the BCR/ABL-1 rearrangement, it is classified as CML, regardless of the LAP score. Just as a low LAP score does not make a diagnosis of CML, a normal or high LAP score doesn’t exclude it.

Current understanding of the pathogenesis of CML and PNH allows utilization of much more sensitive and specific tests for diagnosis. For suspected CML, BCR/ABL, Translocation 9;22, Fluorescence In Situ Hybridization (FISH) should be ordered. For suspected PNH, PI-Linked Antigen is the best laboratory test. Both tests are available through Saint Luke’s Regional Laboratories in conjunction with Mayo Medical Laboratories.

Flow Cytometry Interpretive Reports

Effective 12/14/09, St. Luke’s Regional Laboratories will change the reporting format for comprehensive leukemia/lymphoma flow cytometry panels. The report will include detailed qualitative and quantitative analysis of populations of interest (lymphoid cells, blasts, or other cells) with a diagnostic interpretation. All markers used in analyzing the case will be listed. The current tabulated format of percent positive cells will not be used. This change is in accordance with recommendations of the 2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia (Wood BL et al. Cytometry Part B (Clinical Cytometry) 72B:S14-S22, 2007).

Laboratory Testing for Premature Rupture of Membranes

Preterm premature rupture of membranes (PPROM) refers to rupture of fetal membranes prior to labor in pregnancies of less than 37 weeks. It occurs in 3 percent of pregnancies and is responsible for one-third of preterm births. The classic clinical presentation of PPROM is a sudden gush of clear or pale yellow fluid from the vagina. However, many women describe intermittent or constant leakage of small volumes of fluid or a sensation of wetness within the vagina or perineum. A clinical history suggestive of PPROM should be confirmed by visual inspection or laboratory tests to exclude other causes of wetness, such as urinary incontinence, vaginal discharge, and perspiration.

The best method of confirming the diagnosis of PPROM is direct observation of amniotic fluid coming out of the cervical canal or pooling in the vaginal fornix. If PPROM is not obvious after visual
inspection, the diagnosis can be confirmed by testing the pH of the vaginal fluid with nitrazine paper. Amniotic fluid has a pH of 7.0 - 7.7 compared to the normally acidic vaginal pH of 3.8 to 4.2.

False-negative and false-positive nitrazine results occur in up to 5% of cases (Abe, T. Am J Obstet Gynecol 1940; 39:400 and Davidson, KM. Clin Obstet Gynecol 1991; 34:715). False negative results can occur when leaking is intermittent or the amniotic fluid is diluted by other vaginal fluids. False positive results can be due to the presence of alkaline fluids in the vagina, such as blood, seminal fluid, soap, or some infections.

Another confirmatory test is the presence of arborization (ferning). Fluid from the posterior vaginal fornix is swabbed onto a glass slide and allowed to dry for at least 10 minutes. Amniotic fluid produces a delicate ferning pattern, in contrast to the thick and wide arborization pattern of dried cervical mucus. Well-estrogenized cervical mucus or a fingerprint on the microscope slide may cause a false-positive fern test; false negatives can be due to inadequate amniotic fluid on the swab or heavy contamination with vaginal discharge or blood.

The newest confirmatory test is the AmniSure®, which is a point of care immunochromatographic assay that detects trace amounts of placental alpha microglobulin-1 protein (PAMG-1) in vaginal fluid after rupture of fetal membranes. The analytical sensitivity of the test has been set at 5 ng/mL. PAMG-1 is not detected when fetal membranes are intact because the concentration of PAMG-1 in cervical vaginal secretions is less than 0.25 ng/mL. One visible line means a negative result for amniotic fluid, two visible lines is a positive result, and no visible lines is an invalid result.

Two large studies have assessed the efficacy of AmniSure®. In a study of 203 gravidas suspected of ruptured membranes, the sensitivity and specificity of the AmniSure device were 98.9 and 100%, respectively (Cousins, LM et al. Am J Perinatol 2005; 22:317). Test performance was calculated by comparing AmniSure results against clinical history, nitrazine and ferning results, presence of pooling, sonographic evidence of oligohydramnios, and findings from repeated examinations. Another study of 184 gravidas suspected of ruptured membranes reported a sensitivity and specificity of 98.7 and 87.5%, respectively (Lee, SE et al. Obstet Gynecol 2007; 109:634). Test performance was calculated similar to the first study. The cause of false positive results in three patients was unknown; the possibility of a small leak of amniotic fluid that sealed over could not be excluded.

A false positive result may occur in the presence of bleeding. A false negative result may occur when the sample is taken 12 or more hours after a presumed fetal membrane rupture has occurred. AmniSure® should not be used within 6 hours after the removal of any disinfectant solutions or medicines from the vagina.

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**Change in Acute Cardiac Injury Profile**

For the past eight years, the Acute Cardiac Injury Profile (ACIP) offered by the Saint Luke’s Health System included both CKMB and Troponin I. When this profile is ordered a series of three samples is automatically drawn at 0, 3 and 6 hours.

Last year, Allan Jaffe, a cardiologist at Mayo Clinic published an article entitled, “Requiem for a Heavyweight: the Demise of Creatine Kinase-MB (Circulation 2008;118:2200-06). In this article, he reviewed the reasons that Mayo removed CKMB from its cardiac panel. Essentially, elevated troponin levels are almost completely specific for cardiac injury and have better sensitivity than CKMB. Mayo Clinic decided that CKMB added only cost and confusion.

After careful consideration, the Cardiac EPT of Saint Luke’s Care decided to change acute coronary syndrome (ACS) order sets to only include a troponin drawn at 0, 3 and 6 hours. This change became effective in October. Pre and post procedure orders still include a CKMB.

Saint Luke’s Health System laboratories performed more than 44,000 CKMB in 2008. This change will result in significant cost savings.