Saint Luke's Regional Laboratories
Clinical Laboratory Letter

March 2005

Testing for Insulin Resistance

Insulin resistance leads to a constellation of clinical findings including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, glucose intolerance, and proinflammatory and prothrombotic states. Various experts have referred to this group of abnormalities as the Metabolic Syndrome, Dysmetabolic Syndrome (ICD-9 code 277.7), Syndrome X and Insulin Resistance Syndrome. It is important to recognize this syndrome because it is a risk factor for both cardiovascular disease (CVD) and type 2 diabetes.

The prevalence of insulin resistance is increased in nondiabetic individuals with the following medical history:

- Diagnosis of CVD, essential hypertension, polycystic ovarian syndrome, nonalcoholic steatohepatitis or acanthosis nigricans
- Family history of type 2 diabetes, hypertension or CVD
- History of gestational diabetes or glucose intolerance
- Non-caucasian ethnicity, especially Hispanic and South Asian
- Sedentary lifestyle
- Abdominal obesity
- Age >40 years
- Treatment with corticosteroids, antidepressants, antipsychotics, antihistamines, HIV protease inhibitors

Unfortunately, no single laboratory test is diagnostic of the insulin resistance syndrome. At least 3 professional organizations have published laboratory criteria for the diagnosis of this syndrome including the American Association of Clinical Endocrinologists (AACE), National Cholesterol Education Program's Adult Treatment Panel III (ATP III), and the World Health Organization (WHO) (Circulation 2004;109:433-438 & 551-556 and Endocrine Practice 2003;9:240-52).

Lab Test	AACE	ATP III	WHO
Obesity			
Men	BMI ≥25	>40 in	BMI >30
Women	BMI ≥25	>35 in	BMI >30
Blood Pressure			
Men	≥130/≥85	≥130/≥85	
Women	≥130/≥85	≥130/≥85	
Triglycerides	≥150	≥150	≥150
HDL			
Men	<40	<40	<35
Women	<50	<50	<39
Glucose			
Fasting	110 –125	≥110	110 - 125
2h OGTT	140 - 200		140 - 200
Microalbumin			≥20 ug/min ≥30 mg/g

The major difference between these criteria is in the tests for glucose intolerance. Both the AACE and WHO recommend an oral glucose tolerance test (OGTT), even in patients without an elevated fasting glucose. ATP III does not recommend OGTT in such persons, because they believe that the increased sensitivity does not outweigh the increased cost and inconvenience. WHO includes elevated microalbumin in their diagnostic criteria, but the other 2 organizations do not.

ATP III requires 3 of 5 abnormalities for diagnosis of insulin resistance syndrome. WHO requires evidence of glucose intolerance plus 2 more abnormalities. AACE does not specify a defined number of abnormalities and leaves the diagnosis to clinical judgment.

Even though hyperinsulinemia plays a central role in the pathogenesis of the Insulin Resistance Syndrome, none of the organizations includes measurement of plasma insulin in their diagnostic criteria. The main reason for this omission is that methods to quantify insulin are not standardized and values obtained in different laboratories are not comparable. Also, the absolute difference in values between insulin sensitive and insulin resistant individuals is small and a diagnostic cutoff point has

not been established. Finally, no one has demonstrated that an increase in insulin concentration, by itself, can predict the development of CVD.

Although they are not included in the diagnostic criteria, other laboratory tests may be abnormal in individuals with insulin resistance. Examples include increased plasma uric acid, decreased renal uric acid clearance, increased plasminogen activator inhibitor 1, increased fibrinogen, elevated high sensitivity C reactive protein, and increased WBC count.

PCR Replaces Virus Culture for Skin Lesions

Primary infection with varicella zoster virus (VZV) causes chickenpox, while reactivation of the virus is responsible for varicella zoster, or shingles. The most common clinical manifestation of infection is dermal vesicles. Although viral culture of vesicular fluid has been the standard diagnostic test, the sensitivity is less than 50%.

Traditionally, herpes simplex virus 1& 2 and varicella zoster account for virtually all viruses isolated from skin sources in virology laboratories. Saint Luke's Virology laboratory recovered 38 HSV-1, 18 HSV-2, and 3 VZV viruses from a total of 206 skin lesion specimens submitted for culture in 2004. Parallel testing of 40 skin lesion samples in St. Luke's Molecular laboratory yielded 2 additional VZV viruses that were not detected by culture. PCR specificity for HSV and VZV was100% compared to culture. Published studies have demonstrated that PCR has an increased sensitivity of up to 91%.

Saint Luke's Regional Laboratories replaced conventional herpes virus culture of genital lesions with PCR in June 2003. Effective March 3, 2005 all skin lesion specimens submitted for viral culture will be tested by PCR for herpes simplex virus 1 & 2, and VZV. In addition to enhanced sensitivity, PCR results are available within 1-2 days instead of 3 - 10 days required for cultures. The charge for PCR is comparable and specimen collection is the same as for viral cultures. Vesicular fluid can be collected with a tuberculin syringe and transferred to viral transport media, or vesicles can be unroofed and the base of the lesion swabbed to obtain infected epithelial cells. The test can be ordered as Virus Lesion PCR, and the CPT code is 87798.

More Rapid Mycobacteria

Cultivation of mycobacteria from specimens submitted for AFB culture is challenging due to their slow growth rate, finicky nutritional requirements, and numerous biochemical tests needed for definitive identification. Mycobacteria can take as long as 6 weeks to grow from a specimen. Antimicrobial susceptibility testing requires an additonal 7-10 days to perform after mycobacterial species identification has been made. Saint Luke's Regional Laboratories processes an average of 2400 AFB cultures per year. The majority of isolates are *Mycobacterium avium-intracelluare*, followed by the *M. fortuitum-chelonae* group. Fewer than 10 *Mycobacterium tuberculosis* are isolated per year.

Innovations in technology have enhanced detection & identification of mycobacteria in recent years. For example, SLRL has used an automated liquid media system for incubation of AFB cultures, in addition to traditional solid culture media, since 1999. This system automatically analyzes samples for growth every 60 minutes and has improved recovery time for *M. tuberculosis* by several days. Cultures positive for mycobacterial growth are tested by nucleic acid probes to differentiate *M. tuberculosis*, *M. avium-intracellulare*, and *M. kansasii*. These probes are performed as soon as growth is detected from liquid media, often before growth is visible on solid media. Direct amplified (PCR) testing for *M. tuberculosis* from respiratory samples is also available as a send-out test, with results available within 24 to 48 hours.

The most recent addition to the mycobacteriologists' toolbox is nucleic acid sequencing. This technique is particularly useful to identify mycobacteria species for which nucleic acid probes are unavailable, including rapid-growing mycobacteria species of the *M. fortuitum-chelonae* group. The technique requires isolation of bacterial DNA, amplification, sequencing, and data analysis. There are some limitations to sequencing in that some species within a group cannot be differentiated, for example *M. chelonae* from *M. abscessus*. However, the majority of identifications are available within 48 hours, as opposed to 2-3 weeks required for phenotypic identification through biochemical testing. SLRL began sending isolates suspected to belong to the rapid-grower group to Mayo Medical Laboratories for identification by sequencing in January 2005.