Type 2 diabetes mellitus is a heterogeneous disorder characterized by 2 pathogenic defects, impaired insulin secretion and insulin resistance. Impaired insulin secretion leads initially to postprandial hyperglycemia, and as beta cell function declines further, fasting hyperglycemia ensues. Insulin resistance contributes further to and aggravates the fasting and postprandial hyperglycemia. Other abnormalities associated with decreased insulin secretion and insulin resistance (hyperglycemia, hyperinsulinemia, lipolysis, hyperlipidemia, hypertension, and coagulation defects) contribute to the risk of microvascular and macrovascular disease. When data on diabetic morbidity and mortality are adjusted for the conventional risk factors of obesity, hyperlipidemia, and hypertension, individuals with diabetes mellitus exhibit an increased risk of macrovascular disease most likely related to hyperglycemia.

Control of hyperglycemia may markedly delay or prevent microvascular disease in patients with type 1 and type 2 diabetes mellitus but has not been shown consistently to delay or prevent macrovascular disease. Recent studies have called attention to the role of postprandial hyperglycemia as an important independent risk factor for diabetic complications. Glycated (or glycosylated) hemoglobin (hemoglobin A1c [HbA1c]), which has been used as the index of glycemic control in treatment trials, represents an integration of basal and postprandial glucose levels.

Considerable data from epidemiological and interventional studies have demonstrated that elevated plasma glucose levels correlate with microvascular complications, and the incidence and severity of these complications can be reduced by good glycemic control. The American Diabetes Association recommended specific goals for glycemic control (Table 2). These goals of treatment may need to be modified in elderly patients (those ≥70 years old) because of potential adverse effects from the therapy in this age-group.

### Table 1. Criteria for the Diagnosis of Diabetes Mellitus*

<table>
<thead>
<tr>
<th>Biochemical Action</th>
<th>Normal Goal suggested</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose level ≥126 mg/dL or</td>
<td>&lt;110</td>
<td>80-120</td>
</tr>
<tr>
<td>Symptoms (polyuria, polydipsia, unexplained weight loss) plus casual plasma glucose level ≥200 mg/dL or</td>
<td>&lt;120</td>
<td>100-140</td>
</tr>
<tr>
<td>2-h plasma glucose level ≥200 mg/dL during a 75-g oral glucose tolerance test</td>
<td>&lt;4-6</td>
<td>&lt;7</td>
</tr>
</tbody>
</table>

*Casual is defined as any time of day without regard to time of last meal. Adapted, with permission, from the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.

### Table 2. American Diabetes Association Goals for Glycemic Control*

<table>
<thead>
<tr>
<th>Biochemical index</th>
<th>Normal</th>
<th>Goal suggested</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting/preprandial plasma glucose level (mg/dL)</td>
<td>&lt;110</td>
<td>80-120</td>
<td>&lt;80 or &gt;140</td>
</tr>
<tr>
<td>Bedtime plasma glucose level (mg/dL)</td>
<td>&lt;120</td>
<td>100-140</td>
<td>&lt;100 or &gt;160</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>&lt;4-6</td>
<td>&lt;7</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

*Adapted, with permission, from American Diabetes Association.
Physiology and Pathophysiology

Glucose Metabolism

Normal glucose homeostasis depends on 2 states of activity—the absorptive or postprandial state and the basal or postabsorptive state. After a meal is ingested, the body’s primary requirement is to maintain a normal plasma glucose level. Some but not all of this glucose maintenance is accomplished by the secretion of insulin, which occurs in 2 phases: an acute early phase and a secondary late phase. Insulin promotes cellular uptake of 25% of the glucose load into insulin-dependent tissues (mostly muscle). The remaining 75% of the glucose load is taken up by insulin-independent tissues, brain, splanchnic organs (liver and gut), erythrocytes, and kidneys at a rate proportional to the glucose level.

Progression of Diabetes

Beta cell dysfunction with loss of first-phase insulin secretion occurs early in patients with type 2 diabetes mellitus and results in postprandial hyperglycemia. Peripheral tissue resistance to insulin action (insulin resistance) aggravates the postprandial glycemia. As beta cell function deteriorates with decreasing second-phase insulin secretion, marked hyperglycemia and hyperinsulinemia and increased fatty acid and triglyceride concentrations ensue. In type 2 diabetes mellitus, failure of first-phase insulin secretion with progressive failure of second-phase insulin secretion of the beta cell in conjunction with insulin resistance leads to progression of the disease.

A retrospective analysis of patient medical records from residents of Rochester, Minn, has provided a wealth of longitudinal data on patients with type 2 diabetes mellitus. The incidence of type 2 diabetes mellitus in this community (overall adjusted rates per 100,000 person-years) was 80.1 for obese patients and 45.6 for nonobese patients. The prevalence of retinopathy in people with diabetes mellitus in the Rochester study was 2.6% at the time of initial diagnosis. The subsequent incidence of retinopathy was 17.4 per 1000 person-years among people free of retinopathy at diagnosis. By 20 years after diagnosis of type 2 diabetes mellitus, the cumulative incidence of retinopathy was 30% and 36%, respectively, for obese and nonobese patients. The cumulative incidence of persistent proteinuria 20 years after diagnosis was 24.6% compared with 8.2% at diagnosis. Risk factors for proteinuria included older age at onset of diabetes mellitus, male sex, elevated initial fasting plasma glucose (FPG) level, and the presence of retinopathy and macrovascular disease. The risk of chronic renal failure associated with the presence of persistent proteinuria at the time of diagnosis of diabetes mellitus was increased 12-fold, whereas proteinuria developing after diagnosis was associated with a 10-year renal failure risk of 11%.

Acute Hyperglycemia - Experimental studies of acute hyperglycemia have demonstrated effects on renal and nerve function, retinal perfusion, vasodilation, coagulation factors, and atherogenic vascular disease. Hyperglycemia may be implicated in glomerular hyperfiltration, which precedes diabetic renal disease. Acute hyperglycemia aggravates diabetic nephropathy. Acute hyperglycemia impairs gastrointestinal motility in diabetic patients and in normal subjects. Similarly, acute hyperglycemia has adverse effects on esophageal motility and gallbladder contractility. Acute hyperglycemia with myocardial infarction and stroke is associated with an unfavorable prognosis in diabetic and nondiabetic patients. Improved glycemic control with intensive therapy during and after myocardial infarction has a long-term beneficial effect in patients with diabetes mellitus. Thus, acute hyperglycemia in patients with type 1 and type 2 diabetes mellitus is associated with a myriad of metabolic and biochemical abnormalities that are sustained with persistent hyperglycemia and lead to progression of microvascular and macrovascular disease.

Postprandial Glycemia - Prandial glycemia is a physiologic response to nutrient intake and represents a fluctuation from basal glucose. Postprandial glycemia remains within a rather tight range of plasma glucose levels between 100 and 140 mg/dL. Postprandial hyperglycemia (glucose level >140 mg/dL) may be associated with a higher frequency of diabetic complications. Postprandial hyperglycemia in patients with type 2 diabetes mellitus is secondary to loss of first-phase insulin secretion and insulin resistance and is associated with hyperinsulinemia, which may relate to increased cardiovascular risk. Hyperinsulinemia is more likely a marker for insulin resistance rather than an etiologic factor in diabetic macrovascular disease.

High basal FPG levels are associated with exaggerated postprandial glycemic response. With persistent hyperglycemia, beta cell insulin response is lost over time, and low circulating insulin levels occur, necessitating insulin therapy for restoration of first-phase insulin response and improved glycemic control. There may also be up-regulation of peripheral insulin receptors with insulin therapy, and with improved glycemic control, insulin resistance may decrease. Studies of glycemic control have shown lower all-cause mortality in elderly diabetic patients.
In clinical practice, high glycated hemoglobin levels with accurate satisfactory premeal glycemia most likely reflect postprandial hyperglycemia, which needs to be corrected to improve glycated hemoglobin levels. Several recent studies have indicated that postprandial hyperglycemia may be a better index of glycemic control as measured by glycated hemoglobin than is fasting/premeal basal glucose. Previous epidemiological studies have indicated that postmeal glycemia is an important risk factor for macrovascular disease in patients with type 2 diabetes mellitus.

Studies have shown that the risk of fatal and total coronary heart disease (CHD) increased significantly with increasing postmeal glucose levels. Postmeal hyperinsulinemia is also associated with increased risk of coronary disease. However, postmeal hyperinsulinemia reflects both beta cell dysfunction and insulin resistance and therefore is most likely a marker of the underlying pathophysiology of type 2 diabetes mellitus rather than an etiologic factor. Nevertheless, postmeal metabolic and biochemical perturbations are associated with increased cardiovascular risk.

These studies demonstrate that elevated glucose levels in the fasting and postmeal states are associated with an increased risk of macrovascular complications that can be reduced with improvement in fasting and postprandial glycemia in glycated hemoglobin levels. It is imperative that the management of type 2 diabetes mellitus address both basal FPG control and control of postprandial changes. Therapy therefore must be targeted to maintain normal basal FPG and normal postprandial glucose levels. To accomplish this end, ideal timing for treatment intervention in patients with type 2 diabetes mellitus is just before a glucose load, when the body is most sensitive to the effects of insulin and is best able to produce it and to control hyperglycemia related to insulin resistance. Exogenous stimulation of early insulin secretion suppresses hepatic glucose production, reduces maximum glucose excursions, prevents delayed hyperinsulinemia, and minimizes fluctuations in plasma glucose levels. Reduction of insulin resistance with insulin sensitizing agents maintains basal glucose levels within the desirable range.

**Conclusion**

Tight glycemic control should be the goal in treating patients with diabetes mellitus. A renewed interest in the pathophysiology of diabetes mellitus has provided new treatment options based on different mechanisms of action. Recent data suggest that efforts to achieve target glucose levels with an emphasis on early insulin secretion may provide the best measure for modulating the onset of microvascular and macrovascular complications. Treatment with combination therapy may offer the best therapeutic option. The development of the new therapeutic agents, new mechanisms of insulin administration (intranasal, intrapulmonary, oral, and pump therapy), refinement of beta cell and pancreas transplantation, and the development of artificial beta cells will be welcome additions to the treatment options for patients with type 2 diabetes mellitus.


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**Communiqué Goes On-Line**

Mayo Reference Services is proud to announce the launch of the Communiqué Web site at http://www.mayo.edu/mml/communique.html. The mission of the Communiqué is to serve as an educational instrument, providing information about new testing services and enhancing quality diagnostic patient care through the appropriate utilization of laboratory testing. The site currently houses all of the 2001 and 2002 issues, with separate listings for the issue and the New Test Announcements. The site also includes copies of the 2000 and 2001 indexes for rapid reference.

The Web site also can be accessed from the MML home page at www.mayo.edu/mml/.

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**Monoclonal Protein Study Reporting Change**

Previously, the M Spike portion of Monoclonal Protein Study, Urine #8823 was performed and reported out as a number without units. The reporting field was changed to include units with the value. The M Spike units of measure are mg/24 hour for 24-hour collections and mg/dL for collections of less than 24 hours.
Genotyping for \( \alpha_1 \)-Antitrypsin Deficiency and Change in Specimen Requirements

\( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-antitrypsin) is the most abundant serum protease inhibitor and inhibits trypsin, elastase, and several other proteases. The release of proteases into tissue spaces occurs in response to infections and irritants, and can cause tissue damage unless inhibitors such as \( \alpha_1 \)-antitrypsin are present. Produced primarily in the liver, \( \alpha_1 \)-antitrypsin is transported via the bloodstream to the lungs where it inactivates neutrophil elastase, halting enzymatic activity before any destruction of healthy lung tissue occurs.\(^1\) When \( \alpha_1 \)-antitrypsin is not present, or is present in abnormally low amounts, neutrophil elastase activity is unchecked and the enzyme begins digesting normal lung tissue.

\( \alpha_1 \)-antitrypsin deficiency (A1AD) is an autosomal recessive disorder. Affected individuals develop an inherited form of emphysema. Congenital A1AD is associated with the development of emphysema at an unusually early age and with an increased incidence of neonatal hepatitis, which usually progresses to cirrhosis. Patients who are carriers of the abnormal gene may have reduced levels of \( \alpha_1 \)-antitrypsin, but probably produce enough to protect the lungs provided they do not participate in high-risk activities (eg, smoking, working in a high-contaminate environment). Patients with A1AD and carriers who smoke have a much greater risk of developing emphysema than smokers without A1AD.\(^2\)

Emphysema
Emphysema is a lung disease characterized by loss of lung function. Regardless of the cause, genetic or acquired, the disease results in destruction of lung tissue, chronic overinflation of the lungs, and subsequent loss of oxygen exchange capacity. The American Lung Association estimates that approximately 2.8 million Americans have been diagnosed with emphysema.\(^3\) Of these an estimated 50,000 to 100,000 have A1AD-related emphysema. The severity of the disease makes early identification of affected patients essential, as lifestyle changes and/or medical care may slow or halt the progress of the disease.

\( \alpha_1 \)-Antitrypsin Deficiency and Inheritance
More than 75 \( \alpha_1 \)-antitrypsin forms have been identified based on their migration on an isoelectric gel. Most of these forms contain conservative substitutions, are associated with normal quantitative levels of protein, and function normally. The most common phenotype is M and more than 90% of Caucasians are a homozygous M genotype (having 2 identical genes: MM).

A1AD is caused by the production of variant \( \alpha_1 \)-antitrypsin protein molecules that result in decreased serum levels of \( \alpha_1 \)-antitrypsin activity. The most common deficiency variant \( \alpha_1 \)-antitrypsin types are Z (also called protease inhibitor Z–PIZ) and S (also called protease inhibitor S–PIS). SS and ZZ genotypes are associated with severe disease. A1AD occurs because the Z and S variants of \( \alpha_1 \)-antitrypsin remain in the liver, are not released into the bloodstream, and do not reach the lung. Rarely, a patient does not produce any \( \alpha_1 \)-antitrypsin. This variant is called the null variant and is identified as a Null/Null or PIQ0 genotype.\(^4\) Carriers of A1AD are heterozygotes, having both a deficiency and a nondeficiency allele (eg, MS or MZ).

Other Complications of A1AD
Approximately 12-15% of A1AD patients will experience some level of liver damage. For those born with the ZZ genotype, approximately 10% will develop fatal childhood cirrhosis. One theory proposes that the build-up of a high level of \( \alpha_1 \)-antitrypsin in the liver causes the liver damage that results in infantile hepatitis, cirrhosis, and liver cancer. Another theory proposes that the action is similar to what occurs in the lungs that the normal liver tissue is subjected to attack from neutrophil elastase and other enzymes, and that the low level of circulating \( \alpha_1 \)-antitrypsin is not able to neutralize these enzymes.\(^1\)

Patients who have the null genotype do not produce \( \alpha_1 \)-antitrypsin. These patients are at high risk for emphysema, but do not experience the A1AD-related liver problems.\(^3\) These observations support the theory that the liver disease is a result of the “engorged” hepatocytes (high levels of \( \alpha_1 \)-antitrypsin).

A1AD has also been implicated in the development of ulcerative colitis and Crohn disease.\(^5\) Additionally, studies have linked A1AD carrier status and cigarette smoking with some colorectal tumors.\(^6\) Yang et al, propose that an imbalance between neutrophil elastase and \( \alpha_1 \)-antitrypsin levels predisposed individuals to develop squamous cell lung cancer or bronchoalveolar carcinoma.\(^7\)
Laboratory Testing

**Alpha1-Antitrypsin Concentration**
Determination of α1-antitrypsin levels is the first step in the laboratory investigation of α1-antitrypsin-associated disorders. At MML, this is performed using nephelometry. Patients with A1AD have decreased α1-antitrypsin levels, ranging from 0-70 mg/dL (normal =100-190 mg/dL). Carriers have levels that range from 70-125 mg/dL. It is important to remember that α1-antitrypsin is an acute-phase reactant protein; therefore, levels normally increase in response to a variety of inflammatory processes. In such situations, levels in carriers may rise to within the normal range. However, the severely affected A1AD patient will show no significant response in α1-antitrypsin production.

**Alpha1-Antitrypsin Genotype**
Once an individual has been identified as having an abnormal α1-antitrypsin level, genotyping (identifying the genetic constitution) can help determine if the low level is due to A1AD and can identify the patient as either a heterozygous (1 normal and 1 abnormal/variant gene) carrier or a homozygous recessive (2 abnormal/variant genes) affected individual. The diagnosis of A1AD determines treatment options and the genotype results may also guide important lifestyle choices.

Smoking and other environmental contaminants can severely affect the rate of disease progression in both affected individuals and carriers. It is generally accepted that exposure to smoke causes oxidation of the α1-antitrypsin molecule, making the individuals functionally deficient. Early identification of individuals at risk allows them to manage their environment and make lifestyle decisions early to slow progression of the disease.

A homozygous ZZ genotype indicates that the patient has a severe deficiency of α1-antitrypsin and may suffer from emphysema at an early age. The homozygous SS genotype expresses as a less severe deficiency than the ZZ genotype. Additionally, patients may be a combination of the 2 deficiency variants (eg, SZ). Such patients may express a deficiency; however, it is less severe than that in the ZZ genotype.

As carriers, MZ and MS patients may have lower α1-antitrypsin levels, but the reduced levels are not associated with clinical disease. The carrier genotype, however, has implications that individuals may want to consider during family planning.

**Testing For Alpha1-Antitrypsin Genotype Replaces Alpha1-Antitrypsin Phenotype**
MML previously utilized isoelectric focusing (IEF) to characterize the serum α1-antitrypsin protein in order to determine phenotype (Alpha1-Antitrypsin Phenotyping, Serum). The phenotyping test has now been replaced by a genotyping test utilizing LightCycler™ technology. Advances with polymerase chain reaction (PCR) techniques have yielded a genotyping test that is:
- Rapid
- Accurate
- Less labor intensive
- More reproducible

In a prospective study comparing genotyping plus α1-antitrypsin levels to phenotyping, of 512 specimens only 10 cases were discordant. Of the 10 discordant cases, 5 were unscorable by IEF, 2 were misphenotyped, 1 was misgenotyped, and 2 were misclassified by both methods. The specimens that were misgenotyped were “flagged” because of the inconsistency with the serum α1-antitrypsin levels. Given the ease of result interpretation and scalability, we recommend Alpha1-Antitrypsin Genotype Profile (#83050) in conjunction with nephelometry (to determine α1-antitrypsin levels), as the preferred method for detection of the Z and S alleles in patient specimens.

With the introduction of this new test, genotyping becomes the primary test performed at MML to identify α1-antitrypsin deficiency alleles in patients with low serum α1-antitrypsin levels or with clinical indications of A1AD. Genotyping by real-time PCR will report out the presence of Z, S, or non-Z/non-S alleles (non-Z/non-S alleles represent normal alleles). The laboratory will perform both PCR to determine genotype and nephelometry to determine serum α1-antitrypsin levels. In cases where the genotype and the serum protein levels do not agree (see Figures 1 and 2), phenotyping is automatically performed to help clarify the genotypic result.

**Specimen Requirement Change**
With the change in testing comes an associated change in specimen requirements. Alpha1-Antitrypsin Genotype Profile (#83050) requires 1.0 mL of serum for nephelometry and an additional whole blood (EDTA) specimen of 3 mL for the genotyping portion of the profile. Both specimens must accompany any request for α1-antitrypsin testing. If only serum is received, the laboratory will be unable to perform genotyping.
Figure 1. Alpha-1-Antitrypsin Testing Algorithm
<table>
<thead>
<tr>
<th>AAT level mg/dL</th>
<th>Genotype</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-190</td>
<td>Non-Z/Non-S</td>
<td>Normal - no further testing</td>
</tr>
<tr>
<td></td>
<td>Z/Non-Z or S/Non-S</td>
<td>Consistent with serum level - carrier status. No further testing.</td>
</tr>
<tr>
<td></td>
<td>ZZ</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td>70-99</td>
<td>Non-Z/Non-S</td>
<td>Consistent with serum level - carrier status. No further testing.</td>
</tr>
<tr>
<td></td>
<td>ZZ</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td>&lt;70</td>
<td>Non-Z/Non-S</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td></td>
<td>ZZ</td>
<td>Results inconsistent - perform phenotyping</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>Consistent with serum level - homozygous recessive. No further testing.</td>
</tr>
</tbody>
</table>

Figure 2. Alpha₁-Antitrypsin Outcomes Chart

References

T-Cell and B-Cell Gene Rearrangement CPT Code Correction
In the June New Test Announcements, the CPT codes for T Cell Gene Rearrangement #83122 and B Cell Gene Rearrangement #83123 were incorrect. In addition, the list fee for one component, T-Cell Gene Rearrangement, Southern Blot (#19026), was incorrect; the correct fee is $369.70. The following CPT codes are suggested.

T Cell Gene Rearrangement #83122
CPT Code Information:
#8874
Path Consult, Limited w/o review of patients medical record
CPT 80500
#19024
T-Cell Rearrangement By PCR
83894x2/Electrophoresis
83901x2/Each multiplex PCR
#19023
Gene Rearrangement Extract/Interp
83891/DNA extract/purify
83912/Interpretation and report
#19026
T-Cell Gene Rearrangement, Southern Blot (if appropriate)
83892x2/ Enzyme digestion
83894x2/ Electrophoresis
83896x2/ Nucleic acid probe, each
83897x2/ Nucleic acid transfer

B Cell Gene Rearrangement #83123
CPT Code Information:
#8874
Path Consult, Limited w/o review of patients medical record
CPT 80500
#19025
B-Cell Rearrangement By PCR
83901x2/Each multiplex PCR
83894x2/Electrophoresis
#19023
Gene Rearrangement Extract/Interp
83891/DNA extract/purify
83912/Interpretation and report
#19027
B-Cell Gene Rearrangement, Southern Blot (if appropriate)
83892x2/ Enzyme digestion
83894x2/ Electrophoresis
83896x2/ Nucleic acid probe, each
83897x2/ Nucleic acid transfer
Coccidioides immitis-Immunodiffusion Method Added

Coccidioides immitis antibody detection is accomplished by complement fixation. Recently an immunodiffusion method was added to the Coccidioides, Antibody, Serum #8295 and Fungal Serology Survey, Serum #8236. Immunodiffusion detects the IgM and/or IgG class antibodies and the addition of this method markedly increases the sensitivity of serologic testing for coccidioidomycosis to over 90% for primary symptomatic cases. The new method detects precipitating antibodies present within 1-4 weeks after the onset of infection. The report now lists separate determinations of IgG and IgM by immunodiffusion.

NTx-Telopeptide Reporting Change

NTx-Telopeptide, Urine #81549 will no longer report a t-score. The t-score was calculated from the transformed Gaussian standard deviates corresponding to the percentiles of the healthy subjects aged 20-40 years. While t-scores were intended to clarify the utility of NTx testing, this method of reporting has caused confusion and will no longer be provided.

Meeting Calendar

Interactive Satellite Programs . . .

- September 17, 2002
  Advances in Wound Healing
  Presenter: Steve Kavros, DPM
  Moderator: Robert Kisabeth, MD

- October 22, 2002
  Bone Marker Assays: Are They Useful for the Diagnosis & Treatment of Osteoporosis?
  Presenter: Lorraine Fitzpatrick, MD
  Moderator: Robert Kisabeth, MD

- November 19, 2002
  HIV Update
  Presenter: Zelalem Temesgen, MD
  Moderator: Robert Kisabeth, MD

- December 10, 2002
  Stroke Prevention and Management
  Presenter: David Wiebers, MD
  Moderator: Robert Kisabeth, MD

Upcoming Education Conferences . . .

- Practical Spirometry
  September 13-14, 2002
  Holiday Inn Chicago City Centre
  Chicago, Illinois

- Quality Issues in Phlebotomy
  October 10-11, 2002
  Mayo Clinic, Siebens Building
  Rochester, Minnesota

  Course Co-Directors: Linda Iverson & Sharon Wiesner

For a complete listing of all the courses offered throughout the year, contact the Mayo Reference Services Education Office at 1-800-533-1710 or 507-284-8742.
HER2 Testing in Patients with Breast Cancer: Poor Correlation Between Weak Positivity by Immunohistochemistry and Gene Amplification by Fluorescence In Situ Hybridization

Edith A. Perez, MD; Patrick C. Roche, PhD; Robert B. Jenkins, MD, PhD; Carol A. Reynolds, MD; Kevin C. Halling, MD, PhD; James N. Ingle, MD; and Lester E. Wold, MD

• **Objective:** To evaluate amplification of the HER-2/neu gene by fluorescence in situ hybridization (FISH) in tumors with weakly positive (2+) immunohistochemical staining.

• **Methods:** A total of 1556 breast tumor biopsy specimens were referred to Mayo Medical Laboratories, Rochester, Minn, for HER2 testing between August and December 2000. Immunohistochemical (IHC) analysis was performed with use of a diagnostic test for the assessment of HER2 overexpression, the HercepTest. The IHC stained slides were interpreted and scored on a scale ranging from 0 to 3+ according to Food and Drug Administration–approved guidelines. All specimens scored as 2+ were also routinely evaluated by FISH with use of a HER-2/neu DNA probe kit (PathVysion). Specimens were determined to be amplified if the ratio of HER-2/neu signals to chromosome 17 centromere (CEP17) signals was higher than 2.0.

• **Results:** Thirty-eight percent of the specimens evaluated with the HercepTest were scored 0, 35% were 1+, 14% were 2+, and 13% were 3+. Of the 216 tumor specimens scored as 2+, 26 (12%) had a high level of HER-2/neu gene amplification, 54 (25%) demonstrated duplication of HER2, 4 (2%) deleted HER-2/neu and/or CEP17, and 123 (57%) had no apparent HER-2/neu anomaly, no apparent CEP17 anomaly, nor apparent single gain (aneusomy) of CEP17.

• **Conclusion:** We recommend that all specimens with a 2+ HercepTest result be evaluated by FISH for HER-2/neu gene amplification. The results of both assays should be considered before making a decision to recommend anti-HER2 therapy.

Mayo Clinic Proceedings 2002;77:148-154

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**Proposed diagnostic algorithm for HER2 testing:**

(NOTE: MML test #81504 HER2/neu (c-erbB2), Immunoperoxidase Stain automatically reflexes to #81954 Fluorescence In Situ Hybridization (FISH) for Detection of HER2/neu Amplification Associated with Breast Cancer when immunoperoxidase score is 2+)

Algorithm reprinted with permission from Perez EA, Mayo Clinic Proceedings 2002;77:153.
Q: Can Mayo provide a numeric risk calculation for Down syndrome in twin pregnancies?

A: We are unable to provide a numeric (1/X) risk estimate for Down syndrome in twin pregnancies. The population of twin pregnancies screened where one fetus has Down syndrome and the other does not is significantly small as to not allow for extrapolation of results to the general population of women carrying twin pregnancies. Additionally, it is impossible to determine the amount of each analyte Alpha-Fetoprotein (AFP), Human Chorionic Gonadotropin (hCG), etc contributed by each fetus. The screen-positive or screen-negative estimate is derived by dividing the multiple of median (MoM) value for each marker by the corresponding median MoM in unaffected twin pregnancies. A pseudo-risk is calculated and compared with the risk cut-off to classify the result as screen-positive or screen-negative. Neither the true risk nor the detection rate can be calculated, because the distributions of the serum markers in twin pregnancies with Down syndrome are not known.

Q: Does Mayo provide a numeric risk calculation for neural tube defects (NTD)?

A: No. The risk for a woman to have a child with a neural tube defect is dependent not only on the level of AFP found in the maternal serum screen, but also geographic location, race, family history, maternal conditions such as diabetes and epilepsy, and the use of certain medications. MML provides a high volume of testing both nationally and internationally. Due to the difficulty of obtaining accurate detailed information regarding the factors listed above, we feel the patient’s obstetric provider can more accurately assess the patient’s pretest risk and incorporate this with the level of AFP and appropriate follow-up testing.
ANCA Testing Update and Algorithm

MML has introduced a new panel for the evaluation of patients who have not been previously diagnosed with an antineutrophil cytoplasmic antibodies (ANCA)-associated disease. An algorithm outlining the performance of this panel is provided on the back of this page. Clients wishing to order the complete ANCA panel will need to order Antineutrophil Cytoplasmic Antibodies Vasculitis Panel, Serum #83012. (See separate New Test Announcement #83012 for test specific information.)

Tests for the evaluation and monitoring of patients who have already been diagnosed with an ANCA-associated disease have been changed to allow the most appropriate ordering options. Previously, when Cytoplasmic Neutrophil Antibodies, Serum #9441 yielded positive pANCA results, Myeloperoxidase Antibodies, Serum #80389 was performed at an additional charge. This practice has been discontinued. Now, when test #9441 is ordered, the laboratory will test and report pANCA results and cANCA results (including the titer if positive for cANCA) only. For patients with Myeloperoxidase (MPO)-associated vasculitis, the appropriate test to order is #80389. For patients who have already been diagnosed with Wegener’s granulomatosis, the appropriate test for monitoring is Cytoplasmic Neutrophil Antibodies, Serum #9441.

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Test Name</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>83012</td>
<td>Antineutrophil Cytoplasmic Antibodies Vasculitis Panel</td>
<td>PR3 ANCA</td>
</tr>
<tr>
<td></td>
<td>(See August New Test Announcement)</td>
<td>MPO ANCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If positive, cANCA and pANCA will be run</td>
</tr>
<tr>
<td>82965</td>
<td>Autoantibodies to Proteinase 3</td>
<td>PR3 ANCA</td>
</tr>
<tr>
<td></td>
<td>(See August New Test Announcement)</td>
<td></td>
</tr>
<tr>
<td>9441</td>
<td>Cytoplasmic Neutrophil Antibodies</td>
<td>cANCA and pANCA</td>
</tr>
<tr>
<td>80389</td>
<td>Myeloperoxidase Antibodies</td>
<td>MPO</td>
</tr>
</tbody>
</table>

See reverse side for testing algorithm.
ANCA Panel for Vasculitis

ANCA Panel for Vasculitis #83012
- Autoantibodies to Proteinase 3 #82965
- Myeloperoxidase Antibodies #80389
- Cytoplasmic Neutrophil Antibodies #9441

Both MPO & PR3 - Negative.
Cytoplasmic Neutrophil Antibodies #9441 cancelled
- reported as "Test Not Performed" (TNP).

MPO >5.0 EU/mL

PR3 >5.0 EU/mL

Cytoplasmic Neutrophil Antibodies #9441
Performed and reported

Reported as MPO and PR3 - Negative STOP
Calcitonin Test Update

The method for Calcitonin, Serum #9160 was changed from a radioimmunoassay (RIA) after cartridge extraction method to a 2-site chemiluminescence immunoassay method. This change has resulted in changes to several fields, including specimen type, specimen requirements, and the reference values. This method change also results in improved service and turnaround times as the test is now run Monday through Saturday and reported the same day.

**New Specimen Required:**
Draw blood in a plain red-top tube(s). Spin down and send 0.8 mL of serum frozen in a plastic vial.

**Previous Specimen Required:**
Draw blood in a green-top (heparin) tube(s) from a fasting patient. Spin down immediately in a refrigerated centrifuge and transfer 5.0 mL of heparinized plasma to plastic vial(s). Freeze specimen immediately. Send frozen plasma on 5 lbs. of block dry ice. For diagnosis of medullary carcinoma, the Short Calcium Infusion Test is used and blood collected at 0, 5, and 10 minutes. Mayo Medical Laboratories should be consulted for additional information on the Short Calcium Infusion Test, including necessary precautions.

**New Reference Values:**

<table>
<thead>
<tr>
<th>Test</th>
<th>Basal</th>
<th>Peak Calcium Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males:</td>
<td>&lt;16 pg/mL</td>
<td>≤130 pg/ml</td>
</tr>
<tr>
<td>Females:</td>
<td>&lt;8 pg/mL</td>
<td>≤90 pg/ml</td>
</tr>
</tbody>
</table>

**Previous Reference Values:**

- **BASAL**
  - Maximum reference value has not exceeded
    - Males: ≤19 pg/mL
    - Females: ≤14 pg/mL
- **CALCIUM INFUSION (2.4 mg of calcium/kg)**
  - Maximum reference value has not exceeded
    - Males: ≤190 pg/mL
    - Females: ≤130 pg/mL
- **PENTAGASTRIN INJECTION (0.5 µg/kg)**
  - Maximum reference value has not exceeded
    - Males: ≤110 pg/mL
    - Females: ≤30 pg/mL

**Clinical:**
In the normal physiological situation calcitonin is a polypeptide hormone secreted by the parafollicular, or C-cells, of the thyroid gland. The main action of calcitonin is the inhibition of bone resorption by regulating the number and activity of osteoclasts. Calcitonin is secreted in direct response to serum hypercalcemia and may prevent large oscillations in serum calcium levels and excessive loss of body calcium. However, in comparison to parathyroid hormone (PTH) and 1,25 OH-vitamin D, the role of calcitonin in the regulation of serum calcium in humans is minor and measurements of serum calcitonin levels is therefore not useful in the diagnosis of disorders of calcium homeostasis.

Malignant tumors arising from thyroid C-cells (medullary thyroid carcinoma [MTC]) usually produce elevated levels of calcitonin. MTC is an uncommon malignant thyroid tumor, comprising less than 5% of all thyroid malignancies. Approximately 25% of these cases are familial, usually appearing as a component of multiple endocrine neoplasia type II (MENII, Sipple syndrome). MTC may also occur in families without other associated endocrine dysfunction, with similar autosomal dominant transmission as MENII, and is then called familial medullary thyroid carcinoma (FMTC). Mutations in the RET proto-oncogene are associated with MENII and FMTC.
Other neuroectodermal endocrine tumors, particularly islet cell tumors, may also produce calcitonin, but do so much less frequently. Additionally, calcitonin levels may be elevated in some patients with cancer of the lung, breast, or pancreas; intestinal, gastric, or bronchial carcinoids; patients with chronic renal failure; Zollinger-Ellison syndrome; pernicious anemia; in pregnant females at term; and in newborns.

Clinical:
(continued)

Useful For:
- Diagnosis and follow-up of MTC
- Adjunct to diagnosis of MENII and FMTC
- Occasionally useful in the diagnosis and follow-up of islet cell tumors

Interpretation:
Elevated basal and/or calcium-stimulated calcitonin indicates MTC.

Although most patients with sporadic MTC have high basal plasma calcitonin levels, 30% of those with familial MTC or MENII have normal basal levels. In the past, these individuals may have required a calcium infusion provocative test (short calcium infusion with blood sampling at 0,5, and 10 minutes) to demonstrate the abnormality. Mutation screening (test #80573 Multiple Endocrine Neoplasia Type 2A and Familial MTC, Molecular Analysis, Blood) of RET has largely superseded calcium infusion provocative testing. Calcium infusion tests are now only necessary in suspected familial cases belonging to one of the 5-10% of MEN/FMTC families without detectable RET mutations. For these rare cases the Mayo Clinic Endocrine Testing Unit should be consulted for additional information on the Short Calcium Infusion Test, including necessary precautions.

Following surgical therapy for medullary thyroid carcinoma, serum calcitonin levels in completely cured cases fall, over a variable period of several weeks, into the undetectable range. Persistently elevated postoperative serum calcitonin levels usually indicate incomplete cure. The reasons for this can be locoregional lymph node spread or distant metastases. In most of these cases, imaging procedures are required for further work-up. Those individuals who are then found to suffer only locoregional spread may benefit from additional surgical procedures. However, the survival benefits derived from such approaches are still debated.

A rise in previously undetectable or very low postoperative serum calcitonin levels is highly suggestive of disease recurrence or spread and should also trigger further diagnostic evaluations.

Cautions:
- Not useful for evaluating calcium metabolic diseases.
- False elevated values may occur in serum from patients who have developed human antimouse antibodies or heterophilic antibodies.

Clinical Reference:

Methods:
The Nichols Advantage Calcitonin assay is a 2-site chemiluminescence immunoassay for the measurement of calcitonin in human serum. The patient sample is incubated simultaneously with a biotinylated-mouse calcitonin antibody and a second monoclonal mouse antibody labeled with an acridinium ester. Following incubation, streptavidin-coated magnetic particles are added to the reaction mixture to bind calcitonin-antibody complexes. The washed magnetic particles are transported into a luminometer where a chemiluminescence reaction is initiated. The amount of light generated is directly proportional to the concentration of calcitonin in the specimen. (Package Insert: Nichols Advantage Calcitonin: Chemiluminescence Immunoassay for the quantitative determination of Calcitonin in human serum. Nichols Institute Diagnostics, San Juan Capistrano, CA)