ZAP-70: A New Prognostic Marker For Chronic Lymphocytic Leukemia.

B cell chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, responsible for more than 5000 deaths annually in the United States. The clinical course of CLL varies; some patients have an indolent course with no need for therapy, while others succumb to the disease within a few years. It has recently been shown that cases can be divided into two subgroups based on the presence or absence of somatic mutations in the immunoglobulin heavy chain variable region (IgVH) genes of the leukemic cells. Patients with mutated IgVH genes (50-70% of patients) fare much better than those with unmutated IgVH genes, with median survivals being more than 24 years and 6 to 8 years respectively.

Since knowledge of IgVH mutational status is of considerable value in assessing prognosis in CLL, and since direct analysis of IgVH genes is technically demanding and expensive, a surrogate marker for IgVH mutational status has been actively sought. Expression of CD38 by CLL leukemic cells was initially proposed as a surrogate marker for IgVH mutational status, however subsequent studies failed to confirm CD38 as an independent and consistent prognostic factor. ZAP-70 is a signaling-associated enzyme normally present on T cells. Recently, gene expression profiling has shown that ZAP-70 is also expressed in IgVH unmutated CLL, and is an excellent discriminator of the two prognostic sub-groups of this disorder.

In a recent study of 56 patients with B cell CLL (New Engl J Med 2003;348:1764-75) expression of ZAP-70 was studied in peripheral blood samples by several methods including flow cytometry. The results were correlated with IgVH mutational status and clinical outcome over a median follow-up period of 63 months. IgVH was found to be unmutated in all patients whose leukemic cells were 20% or greater positive for ZAP-70, while IgVH was mutated in 21 of 24 patients with less than 20% ZAP-70 positive leukemic cells. The sensitivity and specificity of a ZAP-70 value greater than 20% for unmutated IgVH were 91% and 100% respectively. Furthermore, higher ZAP-70 values were associated with poorer prognosis of the disease. Patients with Binet stage A CLL who had ZAP-70 values of 20% or greater had more rapid progression and poorer survival than those with ZAP-70 less than 20%. Median survival was 90 months in the former group and not reached in the latter. The level of ZAP-70 did not change over time in sequential samples from 30 patients. In another recent study of 107 patients with CLL, ZAP-70 expression (assayed by methods other than flow cytometry) correctly predicted IgVH mutational status in 93% of patients (Blood 2003;101:4944-4951). Once again, ZAP-70 levels correlated with prognosis. Median time to treatment in patients with high and low ZAP-70 expression was 6.4 years and greater than 10 years respectively.

In conclusion, assay of ZAP-70 in CLL cells by flow cytometry is a simple and reliable surrogate method for determining IgVH mutational status of the disease, and can be used as a prognostic marker, with potential importance in regard to patient management. Starting in September, Saint Luke’s Regional Laboratories will include ZAP-70 as an additional marker in the standard flow cytometry panel for diagnosis of CLL. An interpretive comment will be added in each case of CLL regarding the ZAP-70 value and its prognostic significance.

Modification of Antiphospholipid and Hypercoagulability Panels

In order to comply with the most common ordering practices of our clients, minor modifications will be made to nomenclature and content of the antiphospholipid and hypercoagulability panels, starting in September.

Antiphospholipid I panel includes only coagulation tests for diagnosis of lupus anticoagulant. Indications for this panel include investigation of an unexplained APTT prolongation, or follow-up of a previously diagnosed or borderline
lupus anticoagulant. Tests include APTT, PT, mixing studies, hexagonal phase phospholipid test, dilute Russell viper venom time and thrombin time. Specific components of the panel will vary according to the results obtained.

Antiphospholipid II panel includes all components of Antiphospholipid I panel plus anticardiolipin IgG and IgM antibodies. This panel is the minimum required for diagnosis of the antiphospholipid antibody syndrome in patients with appropriate clinical features (arterial or venous thrombosis or complications of pregnancy).

Antiphospholipid III panel includes the Antiphospholipid II panel plus an assay for anti-beta-2-glycoprotein I, an antibody more closely associated with clinical features of the antiphospholipid antibody syndrome than the presence of anticardiolipin antibodies. This panel offers a more comprehensive approach to diagnosis of the syndrome.

A new Genetic Hypercoagulability panel will be offered which includes only PCR assays for the two most common hereditary causes of thrombosis, factor V Leiden, and prothrombin gene mutation.

The Hypercoagulability I panel is appropriate for laboratory diagnosis of the most common and well-defined hereditary and acquired hypercoagulable disorders. It includes the Antiphospholipid III panel plus activated protein C (APC) resistance, factor V Leiden (if APC resistance is abnormal), prothrombin gene mutation, homocysteine, protein C activity, free Protein S antigen, and antithrombin.

The new Hypercoagulability II panel includes all the above plus a factor VIII activity assay. An elevated factor VIII activity level (>150%) is an independent, common risk factor for venous thrombosis. It should be kept in mind that factor VIII is an acute phase reactant, which limits usefulness of this assay immediately after an acute event.

Thyroglobulin & anti-Thyroglobulin

Thyroglobulin (Tg) is a large glycoprotein that is synthesized in the thyroid follicles and serves as the precursor for thyroid hormone (T4). The primary use of serum Tg measurement is as a tumor marker for thyroid cancer.

A preoperative serum Tg level has no diagnostic or prognostic significance by itself, but does document that thyroglobulin can be followed postoperatively as a tumor marker. Approximately two thirds of patients with thyroid cancer have an elevated preoperative serum Tg level. Preoperative specimens should either be drawn before FNA or more than 3 weeks afterwards. The sensitivity of postoperative serum Tg is highest when the tumor is <2 cm and the preoperative serum Tg value is high.

Following surgery, serum Tg concentrations fall rapidly with a half-life of 2 to 4 days. Any Tg released from surgical margins should disappear within the first two months after surgery. Since the thyroid remnant left after near total thyroidectomy typically approximates 2 grams of tissue, a serum Tg concentration <2 ng/mL is expected when the patient has undergone successful near-total thyroidectomy and has serum TSH maintained below 0.1 mU/L. Tumor recurrence is usually associated with a progressive rise in serum Tg.

Approximately 20% of patients with thyroid cancer produce thyroglobulin antibodies (TgAb). Serial serum TgAb measurements may be an independent prognostic indicator of cancer recurrence. TgAb need to be measured along with Tg because they may interfere with Tg measurements.

Beginning in September, Saint Luke’s Regional Laboratories will begin performing Tg and TgAb assays. All specimens will be tested for Tg and TgAb. Those specimens lacking detectable TgAb will be reported. Specimens with TgAb will be forwarded to the University of Southern California for Tg measurement by RIA, because this method is less prone to interference by TgAb. Turnaround time will improve for specimens without TgAb from the current average of 3 weeks to 3 days.

Reference ranges will change as follows.

<table>
<thead>
<tr>
<th>Test</th>
<th>Tg</th>
<th>TgAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>3 – 40 ng/mL</td>
<td>&lt;1.0 U/mL</td>
</tr>
<tr>
<td>New</td>
<td>1.6 – 55 ng/mL</td>
<td>0 – 40 IU/mL</td>
</tr>
</tbody>
</table>

Specimen requirement is one red top or SST tube of blood. CPT code for is 84432 for Tg and 86800 for TgAb.