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Feature

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of Chronic Lymphocytic
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Current Approach to Diagnosis and Management of Chronic Lymphocytic Leukemia

The diagnostic algorithm, prognostic tools, supportive care measures, and treatment of chronic lymphocytic leukemia (CLL) are changing rapidly. Before the advent of automated instruments for performing blood cell counts, CLL was typically diagnosed when patients presented with symptoms of lymphadenopathy, cytopenias, or infection. The diagnosis of CLL was based primarily on peripheral blood lymphocyte morphology with limited ability to distinguish CLL from the leukemic phase of other subtypes of non-Hodgkin lymphoma. Clinical staging (Rai or Binet) was the limit of prognostication, and “watchful waiting” was the cornerstone of management. CLL was believed to be a disease of elderly persons that was characterized by an indolent course, and most clinicians counseled patients that they would likely die of causes unrelated to CLL before they would require treatment. The infectious complications, increased risk of autoimmune disorders, and increased risk of secondary malignancy associated with CLL were less well defined and clinically underappreciated. The foundation of treatment, monotherapy with alkylating agents, was reserved for patients with advanced-stage disease and was characterized by a low complete response rate.

Nearly all these paradigms have changed. Immunophenotyping, flow cytometry, and cytogenetic evaluation allow increased diagnostic precision and distinguish CLL from mantle cell lymphoma, hairy cell leukemia, splenic marginal zone lymphoma, and peripheral T-cell malignancies. Recent reports state that 50% of patients with early-stage CLL experience rapidly progressive disease, require therapy, and have a median survival period that is significantly shorter than suggested by the original publications of the Rai and Binet

staging systems. Most patients diagnosed as having CLL, including the majority of patients with early-stage disease, die of CLL or CLL-related complications. Novel therapeutic strategies, including monoclonal antibodies, combination chemotherapy, and, for selected patients, stem cell transplantation, have dramatically improved response rates and are likely to lead to improvements in overall survival. New laboratory tests can identify some patients with early-stage CLL at high risk of early disease progression, and clinical trials of treatment of these patients are under way.

Although these advances have increased the importance of early hematology consultation at diagnosis, the burden of diagnosis, supportive care, and appropriate referral is the responsibility of the primary care provider. We summarize the recent advances in diagnosis, prognostic tools, and supportive care measures relevant to the primary care provider of patients with CLL.

Diagnosing CLL—The Modern Approach To Evaluating Lymphocytosis

Chronic lymphocytic leukemia, a clonal disorder of mature B cells, is one of the most common lymphoid malignancies in the United States. CLL affects more than 100,000 Americans, with an estimated 15,000 new diagnoses per year. In the new World Health Organization classification, CLL and small lymphocytic lymphoma are considered a single disease entity (CLL). The median age range at diagnosis is 60 to 68 years with a male preponderance (ratio of women to men, approximately 1:1.8). The clinical manifestations of CLL include fever, night sweats, weight loss (>10% body weight),

fatigue, frequent infections, organomegaly (splenomegaly, hepatomegaly), lymphadenopathy, autoimmune complications, and symptoms related to cytopenias (anemia, thrombocytopenia).

With the advent of automated instruments for performing blood cell counts, CLL in most patients is diagnosed incidentally when an unexpected elevation of the absolute lymphocyte count (ALC) is discovered on a CBC. The diagnosis of CLL should be considered any time a patient presents with an ALC greater than $5.0 \times 10^9/L$ without clear etiology. The differential diagnosis of lymphocytosis is broad and includes a number of malignant and reactive conditions. Before making a diagnosis of CLL, reactive causes of lymphocytosis and the leukemic phase of other lymphoproliferative disorders, particularly mantle cell lymphoma, must be excluded. The optimal evaluation and management of individuals with a persistent ALC of $3.0 \times 10^9/L$ to $5.0 \times 10^9/L$ are unknown. We perform the same diagnostic examination for these patients and

approach patients with a CLL phenotype on flow cytometry in the same manner as patients with early-stage CLL (Rai 0). The differential diagnosis and recommended work-up for patients with lymphocytosis are presented in Figure 1.

Flow cytometry with immunophenotyping is able to establish the diagnosis of CLL in most patients (Table 1). Once the diagnosis of CLL is confirmed, patients should undergo staging and additional laboratory evaluation to help the physician predict prognosis and guide treatment. The staging work-up includes a physical examination (lymph nodes examination, assessment for enlargement of spleen or liver), sequential CBC (to determine lymphocyte doubling time), and examination of the peripheral blood smear. Bone marrow aspirate and biopsy are elective for asymptomatic patients at the time of diagnosis. Bone marrow biopsy is recommended before initiating chemotherapy and may provide additional prognostic information.

Diagnostic criteria	Typical method of testing
1. ALC, $>5.0 \times 10^9/L$	Automated blood counter
2. Clonal B-cell proliferation	Exclusively κ or λ light chain use on flow cytometry Immunophenotype consistent with CLL CD5+, CD19+, CD20+ (dim), CD23+, dim surface immunoglobulin
3. Rule out leukemic phase of other lymphoproliferative disorder (especially mantle cell lymphoma)	Mantle cell lymphoma Flow cytometry†: CD23 dim or absent, CD5+, bright CD20+ FISH: t(11:14)§ Cyclin D1 testing positive Nodal marginal zone lymphoma Flow cytometry†: CD5-, bright CD20 Hairy cell leukemia Flow cytometry†: CD5-, bright CD11c/CD22+, CD19+, bright CD20+, CD103+ TRAP positive Lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia) Flow cytometry†: CD5+/-, CD19+, CD 20+ SPEP-associated monoclonal protein (>2.5 g/dL) Follicular lymphoma (leukemic phase) Flow cytometry†: CD5-, CD19+, CD20+, CD10+/- FISH: t(14:18)§

ALC = absolute lymphocyte count; CLL = chronic lymphocytic leukemia; FISH = fluorescence in situ hybridization; SPEP = serum protein electrophoresis; TRAP = tartrate-resistant acid phosphatase.
†Flow cytometric results must be correlated with careful pathologic assessment of morphologic features.
§Translocation of indicated chromosomes.

Table 1. Diagnostic Criteria for CLL

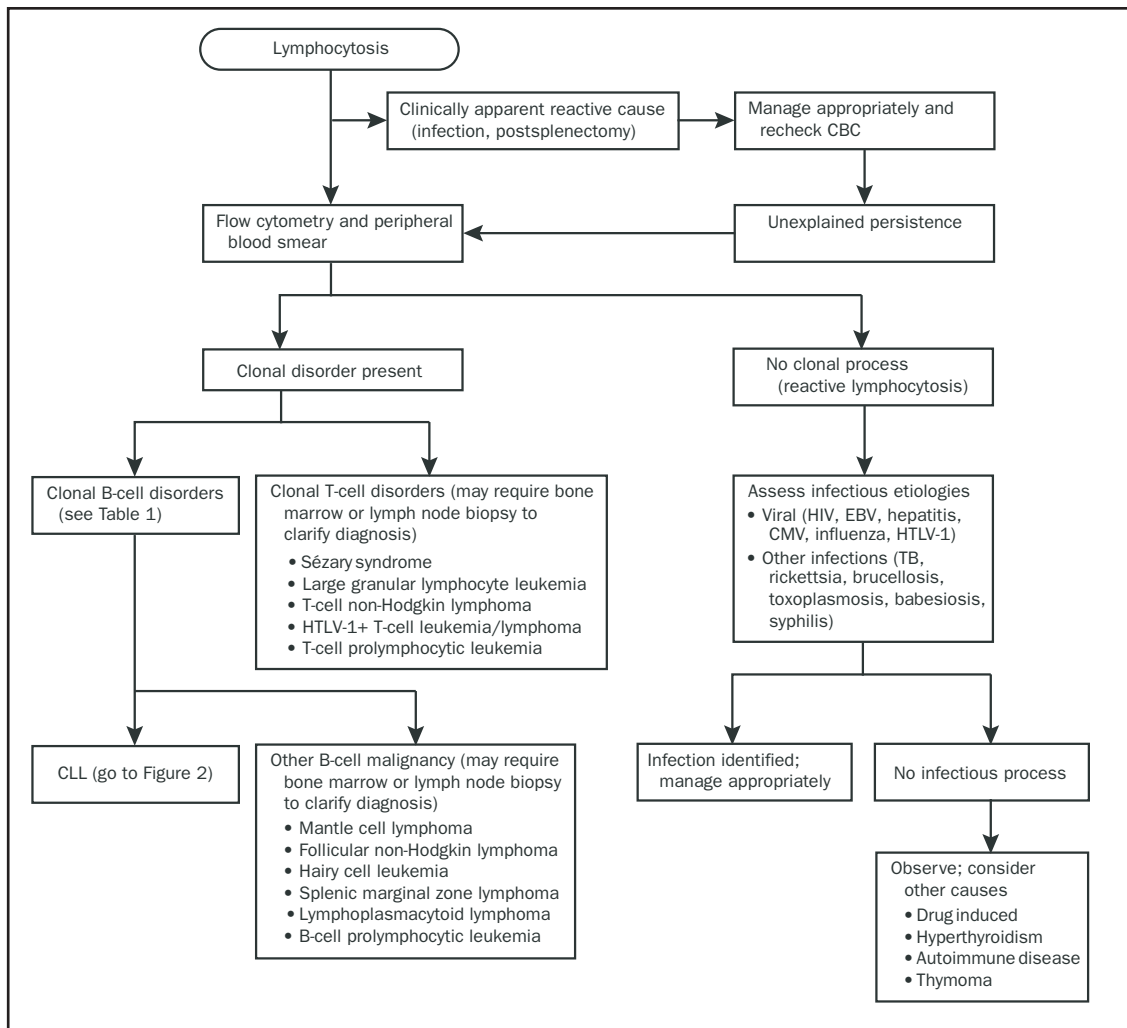


Figure 1. Differential diagnosis and work-up for patients with lymphocytosis.

CBC = complete blood cell count; CLL = chronic lymphocytic leukemia; CMV = cytomegalovirus; EBV = Epstein-Barr virus; HIV = human immunodeficiency virus; HTLV-1 = human T-cell lymphotropic virus type 1; TB = tuberculosis.

Prognostic Tools For CLL— Beyond Staging

Clinical Staging and LDT

The goal of prognostic tools is to enable patients and physicians to predict the natural history of a disease, improve their ability to make treatment decisions, and help patients to plan their lives. For CLL, clinical staging systems were some of the first prognostic tools, and they have been helpful for patient counseling and for grouping individuals with a similar natural history for research purposes.

In 1975, Rai et al developed a clinical “staging” criteria for patients with CLL that separates patients into different prognostic groups based on the presence of lymphadenopathy, organomegaly (spleen and liver), and cytopenias. Rai et al showed a correlation between these clinical stages and prognosis and later modified the 5-stage system to a 3-stage system that categorizes patients as having low risk (original Rai stage 0), intermediate risk (original Rai stages I-II), or high risk (original Rai stages III-IV) disease. The staging criteria and median survival using the Rai staging system are presented in Table 2. Other clinical characteristics,

Rai stage	ALC >5.0 x 10 ⁹ /L	Physical examination		Hemoglobin <10.5 g/dL	Platelets <100 x 10 ⁹ /L	Median survival† (mo)
		Enlarged nodes	Enlarged liver/spleen			
0	+	-	-	-	-	150
I	+	+	-	-	-	101
II	+	+/-	+	-	-	71
III	+	+/-	+/-	+	-	19
IV	+	+/-	+/-	+/-	+	19

ALC = absolute lymphocyte count; + = present; - = absent; +/- = may be either present or absent.
†According to original series by Rai et al.

Table 2. Rai Staging Criteria

including advanced age, male sex, and comorbid disease, also appear to be associated with worse prognosis. Although staging systems are useful clinical tools for grouping patients into low-, intermediate-, and high-risk CLL, there is marked heterogeneity in the clinical progression of disease in patients at similar stage. Approximately 50% of patients with early-stage disease develop more advanced disease and die of CLL or its complications. This highlights the limitations of a prognostic system based solely on markers of disease burden rather than disease biology.

The lymphocyte doubling time (LDT), a clinical measure that addresses the kinetics of cell growth, is calculated by determining the number of months the ALC takes to double. Early studies found that patients with CLL with an LDT of less than 12 months had shorter survival. Because patients with advanced-stage disease (stages III and IV) typically require treatment, the prognostic utility of LDT is most important for patients with early-stage disease who typically are treated by watchful waiting. In one study, the estimated survival for patients with early-stage disease and an LDT of less than 12 months was 66 months, whereas no patients with an LDT greater than 12 months had died at the time of analysis (median follow-up, 48 months). Although other studies have confirmed the prognostic significance of LDT as an independent predictor of disease progression and shorter survival, it is confounded by other factors that cause fluctuations in the ALC, making treatment decisions based on this marker alone problematic. Elevation of the β_2 -microglobulin level, another readily available laboratory test, also suggests worse survival (#9234 [Beta-2-Microglobulin \(Beta2-M\), Serum](#), also performed as part of #5434 [Hematopathology Consultation](#) or #5439 [Surgical Pathology Consultation](#)).

Novel Biologic and Molecular Markers

Historically, clinical stage and LDT have been the most accurate and widely used prognostic tools for counseling patients with CLL. Despite their utility for predicting natural history for populations of patients with CLL, their ability to predict which individuals with early-stage disease will develop advanced-stage disease is imprecise. Recent technological advancements have enabled investigators to search a wider array of biologic and molecular markers to identify differences in disease biology that may predict those patients most likely to develop advanced-stage disease and who may benefit from alternative treatment strategies.

Chromosomal Analysis by Conventional Cytogenetics and Interphase Fluorescence In Situ Hybridization

Chromosomal analysis to identify specific genomic abnormalities has led to breakthroughs in the diagnosis and treatment of several hematologic malignancies, particularly chronic myelogenous leukemia and acute myelogenous leukemia with translocation (15:17). Conventional cytogenetics testing is difficult in CLL because of the small number of dividing leukemic cells. With the development of fluorescence in situ hybridization (FISH), detection of chromosomal abnormalities in nondividing cells became possible, which intensified interest in chromosomal analysis for patients with CLL.

In 2000, German investigators noted the prognostic significance of chromosomal analysis using interphase FISH for patients with CLL. Chromosomal abnormalities were detected in 82% of patients with CLL, with 55% having 13q-, 18% with 11q-, 16% with trisomy 12, 7% with 17p-, and 29% with more than 1 detectable chromosomal abnormality. A hierarchical model constructed after regression analysis identified

Abnormality	Median survival (y)
17p-	2.5
11q-	6.6
12+	9.0
None	9.0
13q-	11.0

CLL = chronic lymphocytic leukemia; FISH = fluorescence in situ hybridization.

Table 3. Prognosis by FISH Result for Patient with CLL.

differences in survival based on the results of FISH testing. The median survival by FISH category after a median follow-up of 70 months is presented in Table 3, which shows a dramatically worse prognosis for individuals with 17p- or 11q- abnormalities. Interphase FISH is a clinically available test for community-based practitioners from Mayo Medical Laboratories as [#83089 Locus and Centromere Anomalies for Chronic Lymphocytic Leukemia \(CLL\), Fluorescence In Situ Hybridization \(FISH\)](#); we recommend FISH testing for all patients with newly diagnosed CLL.

***Ig V_H* Gene Mutational Status**

Until recently, CLL B cells were believed to be derived from the leukemic transformation of “naive” B lymphocytes that had not undergone the somatic mutation of immunoglobulin genes, which occurs when B cells are exposed to antigen in the germinal center of lymph nodes. In the early 1990s, several studies revealed that approximately one half of patients with CLL have somatic mutation of immunoglobulin chains (immunoglobulin variable region of the heavy chain [*Ig VH*]), suggesting that a substantial proportion of CLL clones arise from postgerminal center “memory” B cells.

In 1999, 2 groups of investigators reported on the prognostic significance of somatic mutation of *Ig VH* genes in CLL. When patients with early-stage (Binet A) disease were stratified as *Ig VH*-mutated (>2% difference in nucleotide sequence of *Ig VH* genes from germline) or nonmutated (≤2% difference in nucleotide sequence of *Ig VH* genes from germline), significant survival differences were observed. The median survival for patients with early-stage disease with nonmutated *Ig VH* genes was less than 8 years (95 months), whereas patients with early-stage disease with mutated-type clones had a median survival of greater than 24 years (293 months). Although the prognostic significance of

Ig VH has been confirmed by numerous investigators, *Ig VH* testing is a technically difficult assay that is not currently available for routine use in the United States. Extensive efforts have focused on identifying surrogate markers for *Ig VH* mutational status.

***Ig V_H* Mutational Status and CD38 Expression**

CD38 is a cell surface protein detectable on CLL cells by flow cytometric analysis of peripheral blood. One early report about the prognostic significance of *Ig VH* mutational status described differences in the membrane expression of CD38 on CLL cells and found an association between higher CD38 expression and nonmutated *Ig VH* genes. Subsequent reports substantiated the prognostic significance of CD38 expression but did not validate CD38 expression as a surrogate marker for *Ig VH* mutational status. One study reported an 8-year survival of 92% for patients who were CD38 negative but only 50% for patients who were CD38 positive. The constancy of CD38 expression on CLL B cells is controversial because numerous groups report changes in CD38 status in as many as 10% to 25% of patients with CLL. On balance, it appears that CD38 expression status correlates with poorer prognosis, but its use in individual patients is limited because of variation with time and difficulty in standardizing measurement. CD38 expression is not a reliable surrogate marker for *Ig VH* mutational status.

Zeta-Associated Protein 70 and Other Prognostic Markers

There remains intense interest for developing new, clinically feasible prognostic markers. Gene expression profile analysis (GEP) has identified a restricted set of genes that may discriminate between mutated and nonmutated clones in patients with CLL. One such gene is zeta-associated protein 70 (ZAP-70), a tyrosine kinase normally involved with T-cell signaling, which is expressed differentially by patients with nonmutated CLL clones. Although ZAP-70 appears to be a promising prognostic marker for patients with CLL, numerous issues must be resolved before widespread clinical use.

Summary of Prognostic Tools

Although clinical stage remains the foundation of determining prognosis for patients with CLL, it fails to identify the substantial subset of patients with early-stage CLL who are likely to experience rapid progression to advanced disease. The use of LDT and new molecular-based laboratory tests (FISH, CD38) can identify patients with early-stage CLL who are at high risk of early progression. We routinely perform CD38 testing ([#3287](#)

Leukemia/ Lymphoma Immunophenotyping by Flow Cytometry) and FISH testing (#83089 Locus and Centromere Anomalies for Chronic Lymphocytic Leukemia [CLL]) for all patients with newly diagnosed CLL to improve our ability to counsel such patients, identify individuals who may benefit from a shorter follow-up interval, and identify patients who may be candidates for participation in clinical trials of early intervention. The prognostic work-up for patients with CLL is summarized in Figure 2. Evaluation of *Ig V_H* gene mutational status should be performed if clinically

available at your medical center. In the future, other molecular markers may further improve our ability to identify patients with more aggressive disease.

Patient Follow-Up

Currently, no known curative therapy exists for patients with CLL. Historically, the goals of treatment have been limited to alleviating disease-related symptoms and prolonging survival. Chemotherapy for all (nonselected) patients with early-stage CLL is associated with toxicity and no increase in survival; such treatment is not

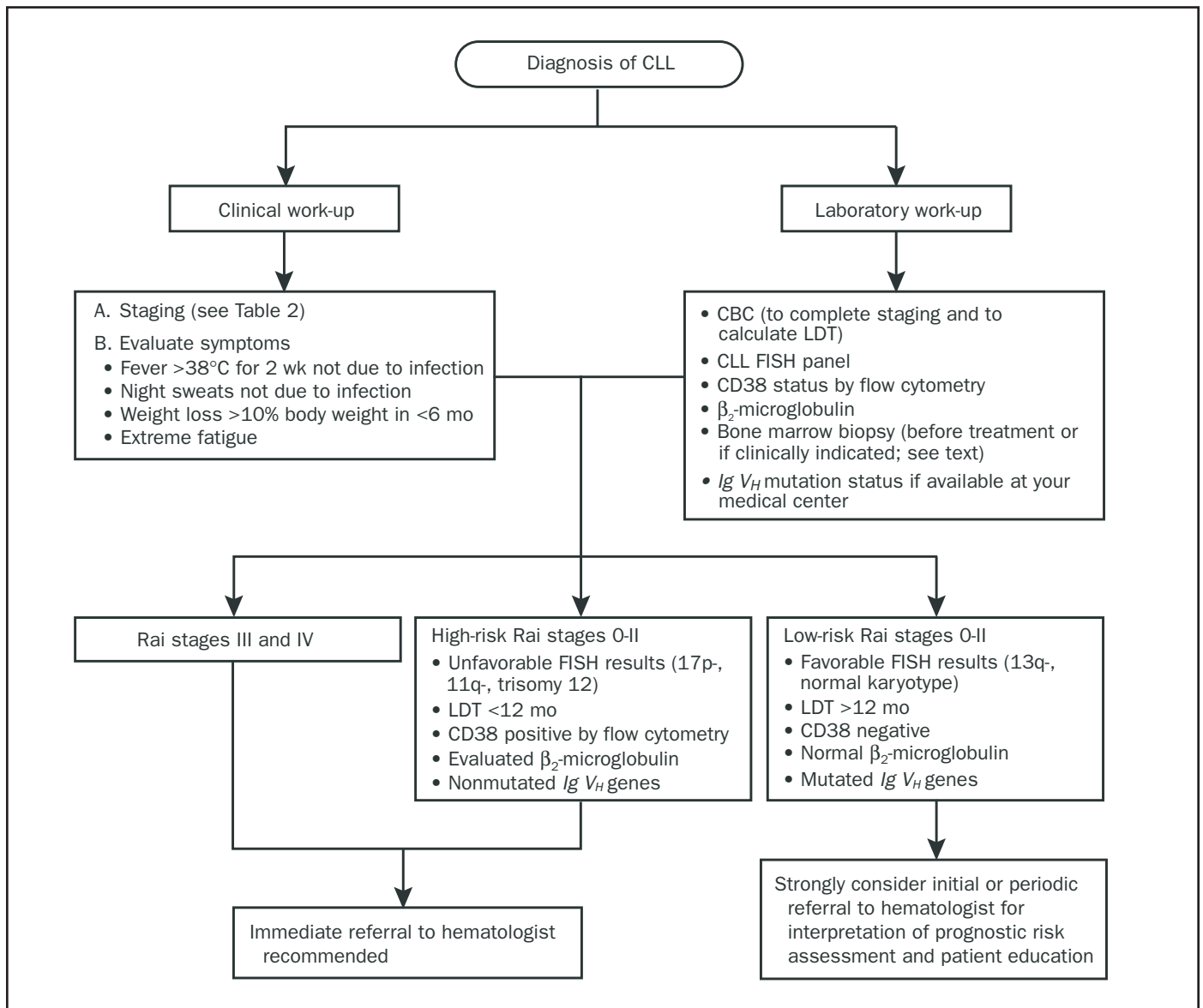


Figure 2. Prognostic work-up for patients diagnosed as having chronic lymphocytic leukemia (CLL). CBC = complete blood cell count; FISH = fluorescence in situ hybridization; LDT = lymphocyte doubling time.

recommended outside of clinical trials. Watchful waiting with active supportive care measures remains the standard of care for all patients with asymptomatic early-stage disease. If immediate treatment is not required, we initially monitor patients every 3 to 6 months for the first year (and every 6-12 months thereafter) to provide supportive care, assess change in lymphadenopathy, calculate LDT, and monitor for cytopenias. We maintain a 6-month follow-up period for individuals with high-risk features (CD38+; 17p- or 11q- by FISH); however, this interval can be lengthened to annual follow-up for patients with clinically stable disease and low-risk features on prognostic testing if patients are aware of the warning signs of disease progression.

Recommendations and Future Directions

Over the past 2 decades, substantial changes have occurred in the precision of diagnosis, stage at diagnosis, accuracy of prognostic tools, treatment options, and supportive care measures for patients with CLL. The primary care provider plays the central role in diagnosis, follow-up, and appropriate referral of these

patients. The fact that more than one half of patients with early-stage CLL will eventually need treatment for CLL, coupled with improvements in our ability to identify and counsel high-risk patients with early-stage disease, suggests that a hematology consultation should be considered for all patients at diagnosis. Although watchful waiting is the cornerstone of management for most asymptomatic patients with early-stage disease, clinical trials for selected patients with markers of biologically aggressive disease are under way. Targeted biologic therapies have expanded treatment options and improved response rates for patients with CLL. Improvements in our understanding of disease-related complications have led to refinements in supportive care guidelines. Application of these advances in diagnosis, prognostication, and supportive care can improve the quality of life for patients with CLL.

Adapted from "Current Approach to Diagnosis and Management of Chronic Lymphocytic Leukemia," *Mayo Clinic Proceedings* 2004;79:388-398. By permission Tait D. Shanafelt MD and Timothy G. Call MD. References and 1 table omitted. The complete article is available online at URL: <http://www.mayo.edu/proceedings/2004/mar/7903cr1.pdf>.

Pernicious Anemia Cascade Test Changes

The **#81499 Pernicious Anemia Cascade** has been assigned a new test number: **#83632 Pernicious Anemia Cascade, Serum**. With this change, each component of the cascade is billed individually, which enables CPT codes to appropriately match with billing.

MML recommends utilization of **#83632 Pernicious Anemia Cascade, Serum** for evaluation for diagnosis of pernicious anemia and diagnosis of vitamin B₁₂ deficiency-associated neuropathy. Historically, many physicians have utilized the Schillings test for evaluating pernicious anemia and vitamin B₁₂ deficiency-associated neuropathy. However, the Schillings test utilizes radioactive methods that have been replaced by newer technologies such as chemiluminescence and tandem mass spectrometry, which provide superior performance and detection without exposing patients to unnecessary radiation. When **#83632 Pernicious Anemia Cascade, Serum** is ordered, the algorithm begins with performance of Vitamin B₁₂ Assay Serum. When indicated, the following tests are performed at an additional charge:

- #9335 Intrinsic Factor Blocking Ab, Serum**
- #80289 Methylmalonic Acid, QN, Serum**
- #8512 Gastrin, Serum**

Thyroid Function Cascade Test Changes

Previously orderable as **#80683 Thyroid Function Cascade, Serum**, this test has been assigned a new test number: **#83633 Thyroid Function Cascade, Serum**. In conjunction with this change, the individually reflexed tests will be billed individually. The test algorithm begins with performance of TSH, Sensitive, Serum. When indicated, the following tests are reflexed at an additional charge:

- #8725 T4 (Thyroxine), Free, Serum**
- #8613 T3 (Triiodothyronine), Total, Serum**
- #81765 Thyroperoxidase Antibodies, Serum**

This change allows CPT codes to be appropriately matched with billing.

Herpes Simplex Virus Test Options Expanded

MML has expanded the number of individual tests available for determination of herpes simplex virus (HSV) antibodies. This change allows those clients who perform HSV IgG on-site to request only the IgM assay, while still offering a comprehensive IgG and IgM option. The new tests offered include:

- #84422 Herpes Simplex Virus (HSV) Types 1 and 2 Antibodies, Serum** (includes HSV Type 1, IgG; HSV Type 2, IgG; and HSV Ab, IgM)
- #84429 Herpes Simplex Virus (HSV) Types 1 and 2 Antibodies, IgG, Serum**
- #80978 Herpes Simplex Virus (HSV) Antibodies, IgM, Serum**

These tests will utilize enzyme immunoassay (EIA) for determination of IgG, and immunofluorescence assay (IFA) for determination of IgM.

With the introduction of these new test options, **#8762 Herpes Simplex Virus (HSV) Types 1 and 2 Antibodies, Serum** has been discontinued.

Fecal Fat Method and Specimen Change

The method for **#8310 Fat, Feces** was changed from a gravimetric method to a nuclear magnetic resonance spectroscopy method. With this method change, the specimen required has been reduced from 10 grams to **5 grams**. This change does not impact reference values.

Lyme Disease Antibody Method Change

The method for the IgM portion of **#9535 Lyme Disease Antibody, Confirmation, Serum** has been changed from an immunofluorescence assay (IFA) to a Western blot assay. This change has also resulted in a change to the reference values. No other aspect of the test has changed.

New Reference Values

- IgG, nonconfirmatory <5 bands
- IgM, nonconfirmatory <2 bands

Previous Reference Values

- IgG, nonconfirmatory <5 bands
- IgM, nonconfirmatory, Negative

Ask



US

Please e-mail your questions to mml@mayo.edu.

Q: What test do I order to measure synthetic glucocorticoids?

A: To measure synthetic glucocorticoids (beclomethasone dipropionate, betamethasone, budesonide, dexamethasone, fludrocortisone, flunisolide, fluorometholone, fluticasone propionate, megestrol acetate, methylprednisolone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide) order [#81035 Synthetic Glucocorticoid Screen, Urine](#). The Endocrine Lab will test for the analytes listed above and quantitate if detected.

Q: Do synthetic glucocorticoids interfere with cortisol urine tests ([#8546 Cortisol, Urine](#) and [#82920 Cortisol, Urine Random](#))?

A: No, with the conversion of the urine cortisol assays (immunoassay and HPLC) to high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), method interference from synthetic glucocorticoids (dexamethasone, fludrocortisone, 6-methyl prednisolone, prednisone, prednisolone, and triamcinolone) is eliminated.

Q: Are 17-ketogenic steroids or 17-hydroxycorticosteroids available in any MML panel? If not, why not and what testing is suggested?

A: 17-Ketogenic steroid and 17-hydroxycorticosteroids assays, which measure cortisol, cortisone metabolites and pregnanetriol, a metabolite of 17-hydroxyprogesterone, are no longer available in any MML panel.

These tests were used primarily to aid in the diagnosis of Cushing's syndrome and adrenogenital syndrome and date back to a time when specific and sensitive assays for the individual analytes were not available. Both tests were performed by oxidation colorimetric reactions, which have been reported to be susceptible to interference by a number of drugs. Because of lack of specificity and susceptibility to interference, both of these tests have been replaced in most laboratories by more specific tests for cortisol, cortisone and 17-hydroxyprogesterone.

It is recommended that urinary free cortisol measurements, [#8546 Cortisol, Urine](#), are used when the purpose of testing is either diagnosis and differential diagnosis of Cushing's syndrome, or as an adjunct in diagnosis and follow-up of conditions of hypocorticalism. At MML this test is performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and is completely free of any interferences, including exogenous noncortisol corticosteroids. MML's LC-MS/MS assay allows simultaneous measurement of cortisone, which can aid in the diagnosis of licorice-ingestion-related hypertension and 11-beta-hydroxysteroid dehydrogenase deficiency.

– Answer continued on next page.

A:

– *Continued*

If testing is aimed at diagnosing adrenogenital syndrome (eg, 21-hydroxylase deficiency) and related disorders, the main former indication for using the 17-ketogenic steroid assay, then the more specific and less interference-prone measurement of serum 17-hydroxyprogesterone is preferred (#9231 [17-Hydroxyprogesterone, Serum](#)). For testing pregnanetriol, a urine metabolite of serum 17-hydroxyprogesterone (#8528 [Pregnanetriol, Urine](#)) done by gas chromatography can be ordered; this method is not influenced by drugs as was the case with the previously offered 17-ketogenic steroid assay.

Q:

What test should I order for diagnostic testing for galactosemia?

A:

Newborn screening programs in all 50 states currently screen for galactosemia. To reduce the risk of life-threatening complications, treatment should be initiated in all infants who screen positive for galactosemia. Follow-up testing via quantitative galactose-1-phosphate-uridyl-transferase enzyme activity (GALT) should be performed quickly to confirm or rule out classic galactosemia. In some cases, the GALT result is not sufficient in distinguishing classic galactosemia (genotype: GG) from Duarte variant galactosemia (genotype: DG). Previously, isoelectric focusing was the preferred method for distinguishing GG patients from DG patients. However, MML recently implemented a new reflex test for galactosemia (#84360 [Galactosemia Confirmation Test, Blood](#)), see New Test Announcement this issue.

The Galactosemia Confirmation Test (#84360) begins with GALT enzyme analysis followed by a reflex to molecular testing. Based upon the enzyme results, specimen results are reported as follows:

- ≥ 18.5 U/g Hgb: The GALT activity in this sample is normal and not consistent with classic galactosemia (GALT deficiency). Molecular genetic testing for Duarte and Los Angeles variants to follow.
- 14.0-18.4 U/g Hgb: The GALT activity in this sample is not consistent with classic galactosemia (GALT deficiency). This result is most consistent with heterozygosity for a mild GALT mutation (ie, Normal (N)/D). However, heterozygosity for a pathogenic mutation in the GALT gene or homozygosity for a mild GALT mutation (ie, DD) cannot be ruled out.
- 8.1-13.9 U/g Hgb: The GALT activity in this sample is reduced; however, not consistent with classic galactosemia (GALT deficiency). Partial enzyme deficiency can be explained by a variety of genotypes.
- 2.1-8.0 U/g Hgb: The GALT activity in this sample is most consistent with the Duarte variant galactosemia (DG). However, heterozygosity for a classic GALT mutation (NG) is also possible. We recommend continuing a galactose-free diet until the diagnosis can be further clarified.
- < 2.0 U/g Hgb: The GALT activity in this sample is most consistent with classic galactosemia (GALT deficiency). However, Duarte variant galactosemia (DG) cannot be ruled out. Treatment with galactose-restricted diet should be initiated immediately.

A patient may have classic galactosemia or be a carrier when the GALT enzyme activity is found to be < 18.5 U/g of hemoglobin. In these cases, the specimen is reflexed to the molecular genetics laboratory for a 6-mutation panel. Patients with enzyme activity ≥ 18.5 U/g of hemoglobin do not have classic galactosemia, but may be carriers for the Duarte (D) or Los Angeles (LA) variants.

– *Answer continued on next page.*

A: – *Continued*
Specimens demonstrating >18.5 U/g activity are reflexed to the molecular genetics laboratory for a 2-mutation panel (D and LA). In a small percentage of cases, [#80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes](#) is necessary to resolve the results of the enzyme and molecular analyses. The decision to reflex to Biochemical Phenotyping (#80341) is made on a case-by-case basis. Clients will be contacted if the laboratory determines phenotype testing is necessary.

Galactose-1-phosphate (G1P), [#80337 Galactose-1-Phosphate \(G-1-P\), Erythrocytes](#), is recommended to assist in monitoring dietary therapy for classic galactosemia (total GALT deficiency), Duarte variant (DG) patients, or rarely, patients with UDP galactose-4-epimerase deficiency. During the initial workup of the patient, this test also can indicate the relative condition of the patient and the need for immediate intervention.

Patients referred to rule out galactosemia (for reasons other than follow-up of a positive newborn screen) should undergo the same testing algorithm.

Q: What test should I order for carrier testing for classic galactosemia?

A: Previously, enzyme analysis in conjunction with isoelectric focusing was the preferred method for detecting carriers for classic or Duarte variant galactosemia. However, MML recently implemented a new reflex test for galactosemia ([#84360 Galactosemia Confirmation Test, Blood](#)), see New Test Announcement this issue.

The Galactosemia Confirmation Test (#84360) begins with enzyme analysis followed by a reflex to molecular testing. Based upon the enzyme activity detected, specimen results are reported as described in the previous question and answer.

Similar to diagnostic testing, the results of enzyme and molecular analyses are not always sufficient to determine carrier status. In a small percentage of cases, [#80341 Galactose-1-Phosphate Uridyltransferase Biochemical Phenotyping, Erythrocytes](#) is necessary to determine whether an individual is a carrier, as molecular analyses do not detect all mutations in the gene. Phenotyping examines the protein allowing for the estimation of genotype, although the exact mutation would not be known. The decision to reflex to Biochemical Phenotyping (#80341) is made on a case-by-case basis. Clients will be contacted if the laboratory determines phenotype testing is necessary.

Q: What is galactokinase deficiency?

A: Galactokinase (GALK) is 1 of the 3 enzymes causing galactosemia when deficient. The other enzymes are galactose-1-phosphate uridyltransferase (GALT) and UDP galactose-4-epimerase (GALE).* GALT is deficient in classic galactosemia, which is the most common (GG occurs in approximately 1:30,000 live births) and clinically most severe form of galactosemia. GALE deficiency is a rare form of galactosemia and can manifest like classic galactosemia. GALK deficiency is characterized by the development of cataracts and galactosemia, but normal GALT activity and galactose-1-phosphate levels. The diagnosis of GALK deficiency can be established by enzyme assay ([#8628 Galactokinase, Blood](#)). GALK deficiency must also be treated with a lactose-free diet.

* *Testing for GALE is not available from MML.*

2004 Education Calendar

Interactive Satellite Programs . . .

Herbal Therapy 2004: Snakes in the Grass (Herb-Drug Interactions Clinicians Need to Know)

September 16, 2004

Presenter: Brent Bauer, MD

Moderator: Robert M. Kisabeth, MD

Markers of Inflammatory Bowel Disease

October 19, 2004

Presenter(s): Henry Homburger, MD and
Edward Loftus, MD

Moderator: Robert M. Kisabeth, MD

The Use of Diagnostic Tests in the Pediatric Age Group

November 2, 2004

Presenter: Robert Jacobson, MD

Moderator: Robert M. Kisabeth, MD

Thyroid Disease – Laboratory Support For Diagnosis and Management

December 7, 2004

Presenter: George Klee, MD, PhD

Moderator: Robert M. Kisabeth, MD

Upcoming Education Conferences . . .

Bleeding and Thrombosing Diseases: The Basics and Beyond

Coagulation Conference and Wet Workshop

August 5-7, 2004

Mayo Clinic, Siebens Building
Rochester, Minnesota

Practical Surgical Pathology

September 16-18, 2004

Mayo Clinic, Siebens Building
Rochester, Minnesota

Practical Spirometry

November 2-3, 2004

Mayo Clinic, Siebens Building
Rochester, Minnesota

Introductory Clinical Mycology

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