A Case-based Review of Chronic Lymphocytic Leukemia

SUSAN J. LECLAIR

LEARNING OBJECTIVES

1. Explain the role of immunochemical testing as a substitute for bone marrow examination in CLL.
2. List the most common immmunophenotype for classic chronic lymphocyte leukemia.
3. Explain the clinical utility of the new prognostic markers for CLL.
4. List the results of the laboratory tests used to routinely evaluate chronic lymphocytic leukemia.

ABBREVIATIONS: CLL – chronic lymphocytic leukemia, IgVH – immunoglobulin heavy chain mutation, ZAP 70 - zeta chain associated protein kinase 70, FISH – fluorescent in situ hybridization, ATM – Ataxia telangiectasia mutated

INDEX TERMS: Chronic Lymphocytic Leukemia, B cell flow cytometry, IgVH mutations

Clin Lab Sci 2013;26(4):182

Susan J. Leclair, PhD, *University of Massachusetts, Dartmouth, MA.*

Address for Correspondence: Susan J. Leclair, PhD, *Department of Medical Laboratory Science, University of Massachusetts , 285 Old Westport Road, Dartmouth, MA 02747-2300, sleclair@umassd.edu*

INTRODUCTION

The diagnosis and treatment of chronic lymphocytic leukemia (CLL) has undergone a profound expansion in scientific and medical understanding in the last decade. Commonly held beliefs of signs and symptoms at time of diagnosis, required laboratory testing, and choices of therapy have all changed.

A 34-year old male medical laboratory science (MLS) student tested his own blood as part of an exercise in the use of an automated instrument. His initial CBC is seen in Table 1. Figure 1 shows an oil immersion magnification view of the lymphocytes on his peripheral blood smear. He agreed to see his primary care physician the next day. The repeat CBC was ordered the next day and the results were essentially unchanged. When questioned the physician said that a viral infection could be the cause. The patient later remarked that he had no obvious signs and symptoms although focused questioning brought out a state of fatigue, night sweats, and a mild viral infection that took over 6 weeks to get over. As is typical with many newly diagnosed patients with CLL, he did not have an anemia or thrombocytopenia. Please see Figure 2.

Table 1. Initial CBC results

TEST	Value	Description	Reference interval
WBC	89.4	$x10^9$/L	4.1 - 11.2
RBC	4.41	$x10^{12}$/L	4.00 – 5.42
Hb	13.7	g/dL	14.0 – 15.9
HCT	41.8	%	42 - 50
MCV	94.8	fL	80 - 100
MCH	31.1	Pg	30.0 – 32.1
MCHC	32.8	g/dL	31.4 – 35.2
RDW – CV	13.5	%	11.5 – 14.0
PLT	222**	$x10^9$/L	150 - 450
MPV	13.4**	fL	7.5 – 10.2
Absolute Differential			
Neutrophils	5.31	$x10^9$/L	4.4 – 7.2
Lymphocytes	81.2*	$x10^9$/L	0.8 – 4.8
Monocytes	1.82*	$x10^9$/L	0 – 1.1
Eosinophils	0.08	$x10^9$/L	0 – 0.4
Basophils	0.22	$x10^9$/L	0 - 0.1
Percentage Differential			
Neutrophils	6*	%	45 - 72
Lymphocytes	92*	%	20 - 44
Monocytes	2	%	1 - 10
Eosinophils	0	%	0 - 4
Basophils	0	%	0 - 1

*Leukocytosis, Lymphocytosis
**Enlarged platelets with abnormal distribution

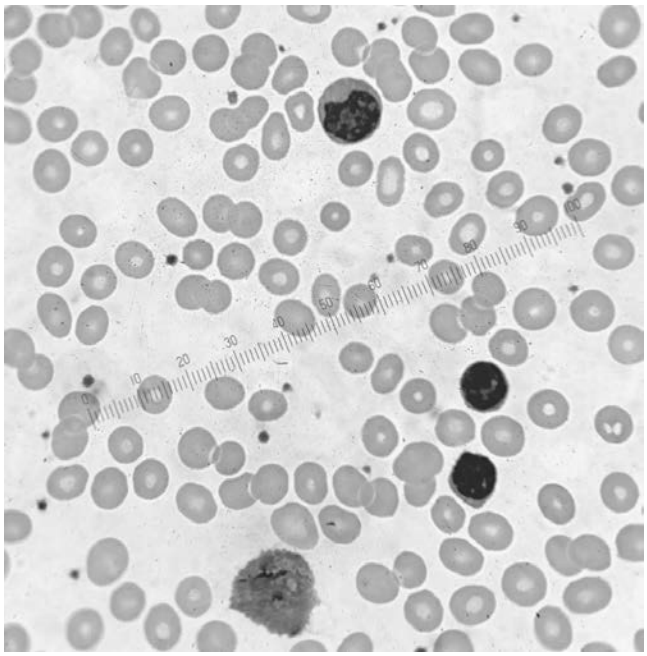

Figure 1. The patient's presentation has the typical monotonous appearance to the lymphocytes and basket cells. A low grade background film suggests an increase in protein in the plasma.

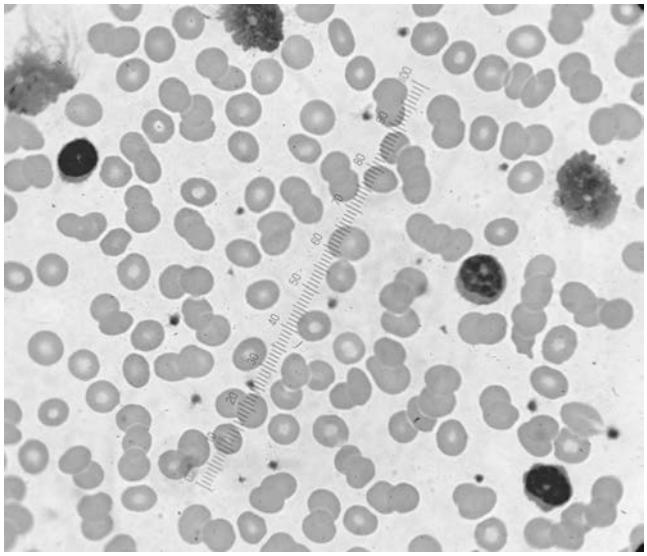

Figure 2. As is commonly seen in early stages of CLL, the patient has normochromic normocytic red blood cells with no anisocytosis or poikilocytosis. No polychromasia is seen. Platelets appear adequate although some were enlarged. Complication seen in more advanced presentations include a hemolytic anemia and/or immune thrombocytopenia purpura.

Family history includes a father who died from a lymphoproliferative (non-Hodgkin lymphoma) disorder in the 1980s. Some studies have suggested that there may be a familial risk of leukemia in relatives of lymphoproliferative disorder patients.[1]

At its base definition, CLL is a monoclonal production of greater than $5x10^9$ B lymphocytes/L mature-looking but dysfunctional lymphocytes in the peripheral blood.[2] While small cells with a dense nucleus and scanty cytoplasm are typically the dominant feature, larger cells and smudge or basket cells are often present. Often red blood cells may be normocytic normochromic and platelets appear adequate in size and granulation.

Chronic lymphocytic leukemia is the most common leukemia of adults.[3] While it is true that the majority of cases are seen in patients over the age of 70, approximately one-third of all CLL cases are found in patients younger than 60 years of age.[4] CLL appears to have gender and ethnicity preference with more males and Caucasians. The traditional belief was that CLL had no significant signs and symptoms at the time of diagnosis. It now appears that patients may have signs and symptoms for an average of 3.5 years prior to diagnosis.[5] Common signs and symptoms include fatigue, night sweats, fever of unknown origin and frequent viral infections. Patients may also have unusual laboratory results including increased white cell and absolute lymphocyte counts, decreased platelet count and increased lactate dehydrogenase. The patient in this situation was a non-insulin dependent diabetic and, while he saw his physician on a scheduled routine for the past three years, no CBC was ever ordered. CAT scans demonstrated small enlargement of numerous axillary nodes.

B lineage derived CLLs constitute approximately 95% of all CLLs.[6] While many presentations appear to be a clonal increase of the common small, resting lymphocytes, these cells are not simple counterparts. While they do share several markers such as CD19, CD20, CD21, CD24, CD40, CD44, CD45R, and sIgM/D, they do not express C3b Complement receptor, LFA-1 or CD22.(see Table 3) B-CLLs also express the T cell associated antigen CD5, and a number of antigens suggesting some form of *in vitro* activation. CD5, a T cell maker can be found during fetal development, and in the adult, can be found in patients with autoimmune disorders and certain *in vitro* activated B lymphocytes.[7]

The presence of CD5, CD19, CD20 and CD23 is the most common presentation of B cell CLL.[8] CD5 can

also be seen on a subset of B cells that are long lived.[9] CD5 suppresses the ability of B cells to respond to B-cell receptor (BCR) signaling and also protects these cells from various apoptotic stimuli.[10] CD19 is a surface receptor whose expression will amplify transmembrane signals and promote cell expansion and survival. When functional, CD19 allows the B cell to respond to fewer antigens.[11] CD20 is expressed on developing B cells from the early pre-B to mature state, although it becomes non-functional in the plasma cell. CD20 activation causes increased tyrosine kinase intracellular signals and regulates intracellular calcium levels.[12] Beta-2-microglobulin levels reflect the level of mitotic and/or metabolically active cells. Immunoglobulin assays provide information concerning the general responsiveness to immune stimulation. The patient's tests results indicated a reasonable immune competency. See Table 2.

Table 2. Patient follow up testing showing results typical of B-cell CLL.

Test	Result	Reference interval
Beta-2-microglobulin	2.6 mg/dL	0.6 – 2.4 mg/dL
IgG	689 mg/dL	700 – 1500 mg/dL
IgA	122 mg/dL	90 – 400 mg/dL
IgM	8 mg/dL	50 – 250 mg/dL
LD	257 µ/L	100 – 200 µ/L
Uric acid	7.9 mg/dL	4. 0 – 8.0 mg/dL

Smudge cells (Basket cells) are seen in situations of cell fragility and are common for patients with CLL. They represent the inherent abnormalities of these cells as they are unable to maintain function and morphology during exposure to EDTA. However unusual they might be, the presence or absence of smudge cells has no prognostic significance.[13] (See Figure 3.)

Molecular Genetic Testing

The availability of genetic testing has further divided CLL into subsets upon which some prognostic determinations can be made. See Table 3. In addition to the CD5, 19, 20 and 23 positivity, the student's workup also included flow cytometric analysis of CD 38; FISH for ZAP70 and IgVH; cytogenetic studies for 13q-, +12, 11q- and genome testing for NOTCH-1. ZAP 70 (zeta chain associated protein kinase 70) is a protein on the membrane of T cells and natural killer cells.[14] It plays a role in T cell activation and I associated with B cell receptors in CLL cells. Expression of ZAP70 in B-cell CLL is a marker for a shorter and more complicated course of the disease. Immunoglobulin variable heavy chain regions (IgVH) is a gene that undergoes rearrangement during maturation. The presence of IgVH suggests that the cell is more mature and in multivariate analyses, IgVH status is a consistent predictor of clinical outcomes with unmutated IgVH negative cells having a significantly worse prognosis.[15] Associated with ZAP70 and IgVH studies is the presence/absence of CD38. CLL cells with high quantities of CD38 are more responsive to BCR signaling. The increased activation appears to encourage proliferation via a pathway that includes ZAP70. This pathway seems to tip the balance between proliferation and apoptosis in favor of proliferation.[16]

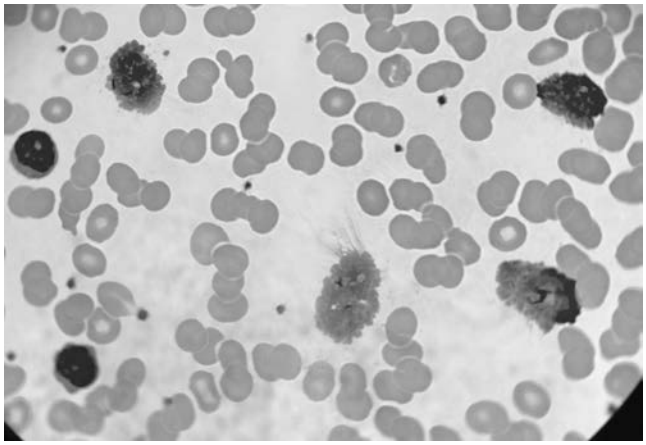

Figure 3. Note the formation of basket or smudge cells. These are found in situations in which fragile cells are incapable of surviving the collection and preparation process for a CBC.

A deletion on the long arm of chromosome 13 is the most common (18-67%) chromosomal abnormality found in CLL.[17] This deletion prevents miRNA15 and 16 from allowing apoptosis, creating cells that can both multiply and accumulate over time.[18] Trisomy 12 is the third most common (11 – 23%) mutation seen in CLL.[19] This mutation is often seen with a cluster of other mutations suggesting that there is increased proliferation and relief from apoptotic signals.[22] NOTCH-1 appears to be part of a cluster of mutations that appear to be constitutively active in B cell CLL.[21] In this case, the molecular testing revealed the presence of non-mutated IgVH, deletion of chromosome 13, and negative results for NOTCH-1, leaving the patient with a mixed but overall good prognostic panel of all but one favorable indicator. See Table 4.

Table 3. Surface membrane antigen patterns seen in CLL compared to the patient's results.[27]

TEST	Expected pattern for CLL	Patient
CD5	+	+
CD19	+	+
CD20	+	+
CD23	+	+
CD27	+	Not performed
CD38	+/- for prognostic use	0
CD40	+	Not performed
CD45	+	Not performed
CD10	0	0
CD14	0	0
CD 34	0	0
Membrane immunoglobulin (mIg)	0	0
Kappa chains	0	0
Lambda chains	0	0

Table 4. Results of genetic testing in this case.

12+	Absent
13-	Present
17-	Absent
ZAP 70	Not performed
IvGH	Non-mutated status
NOTCH-1	Absent
ATM	Absent

Therapy

Patients with active or symptomatic disease or with advanced Binet or Rai stages require therapy. The Binet and Rai classifications concentrate on the involvement of bone marrow, major organs and lymph nodes (see Table 5) while the more current World Health Organization (WHO) system blends patient status with molecular and flow cytometric studies.[22] For physically fit patients, chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab represents the current standard therapy. For frail patients, treatment with an anti-CD20 antibody plus a milder chemotherapy (chlorambucil) is currently established as standard treatment.[23] Fludarabine is a purine analog that inhibits DNA synthesis in actively mitotic cells. Most often red cells will be macrocytic, normochromic while all cell counts will be lowered. Cyclophosamide is an alkylating agent that attaches an akyl group to the quanine found in DNA, inhibiting both replication and transcription.

Table 5. Classical methods of CLL staging

Binet Method	
Clinical Stage I	No anemia or thrombocytopenia and fewer than three areas of lymphoid involvement
Clinical Stage II	No anemia or thrombocytopenia with three or more areas of lymphoid involvement
Clinical Stage III	Anemia and/or thrombocytopenia regardless of the number of lymphoid involvement
Rai Method	
Stage 0	Absolute lymphocytosis greater than 15.0 x 10^9/L with no adenopathy, anemia or thrombocytopenia
Stage I	Absolute lymphocytosis with nodular lymphadenopathy but not hepatosplenomegaly, anemia or thrombocytopenia
Stage II	Absolute Lymphocytosis with hepato or splenomegaly with or without lymphadenopathy
Stage III	Absolute lymphocytosis and hemoglobin less than 11.0 g/dL with or without lymphoid involvement
Stage IV	Absolute lymphocytosis, thrombocytopenia with or without lymphoid involvement

Common side effects include a general myelosuppression, hair loss and significant damage to renal function. Rituximab is a monoclonal therapy directed against CD20. A form of resistance due to tumor cell alterations that in effect shield this marker when confronted with the antibody is seen and changes in the reaction of the body's immune response to the antibody.[24]

At relapse, the initial treatment may be repeated if the treatment-free interval exceeds two years. If the disease relapses earlier, alternative therapies such as bendamustine alone or with rituximab, alemtuzumab, lenalidomide or ofatumumab should be used.[25] Ataxia telangiectasia mutated (ATM) genetic studies should help to inform decisions on potential chemo-resistance.[26] Patients with a del(17p) or TP53 should be considered for an allogeneic stem cell transplant (SCT).

CONCLUSION

Long thought to be a somnolent disease of the elderly, increased awareness of the frequency and subsets of CLL have resulted in better diagnosis and targeted therapy. At this time, it is hoped that the patient in question will be without the need for therapeutic intervention for perhaps a decade. For now, the process

is to watch and wait.

REFERENCES

1. Yuille MR, Matutes E, Marossy A, Hilditch B, Catovsky D, Houlston RS. Familial chronic lymphocytic leukemia: a survey and review of published studies. Br J Haematol 2000;109:794-9.
2. Halleck, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Kipps TJ, et al. Guidelines for the diagnosis and treatment of chromic lymphocytic leukemia: a report from the Internation Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 Guidelines. 2008;111(12):5446-56.
3. Hallek M. Chronic lymphocytic leukemia: 2013 update on diagnosis, risk stratification and treatment. Am J Hematol. 2013 May 30.
4. N.I.H. National Cancer Institution. SEER Stat Fact Sheets: Chronic Lymphocytic Leukemia. Available at http://seer.cancer.gov/statfacts/html/clyl.html#incidence-mortality. Accessed June 10, 2013.
5. http://seer.cancer.gov/statfacts/html/clyl.html. Accessed July 2 2103.
6. Hallek M, Cheson BD, Catovsky D, et al.: Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008;111(12):5446-56.
7. Freedman AS. Immunobiology of chronic lymphocytic leukemia. Hematol Oncol Clin North Am. 1990;4(2):405-29
8. Dighiero G, Hamblin TJ: Chronic lymphocytic leukaemia. Lancet 2008;371(9617):1017-29.
9. Gary-Gouy H, Harriague J, Bismuth G, Platzer C, Schmitt C, Dalloul AH. Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. Blood. 2002;100(13):4537-43
10. Cioca DP, Kitano K. Apoptosis induction by hypercross-linking of the surface antigen CD5 with anti-CD5 monoclonal antibodies in B cell chronic lymphocytic leukemia. Leukemia. 2002;16(3):335-43.
11. Carter RH, Fearon DT. Pillars Article: CD19: Lowering the threshold for antigen receptor stimulation of B lymphocytes. Science 1992;256:105-7.
12. Popoff IJ, Savage JA, Bland J, Johnson, P, Deans LP. The association between CD20 and Src-family tyrosine kinases requires an additional factor. Mol Immunol, 1998;35:207–14
13. Mozaheb Z, Hasanzadeh NazarAbadi MH, Aghaee MA. Chronic lymphocytic leukemia and prognostic factors Asian Pac J Cancer Prev. 2012;13(7):3009-13.
14. Chan AC, Iwashima M, Turck CW, Weiss A. ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. Cell 1992;71(4):649–62.
15. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840–7.
16. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N. CD38 and chronic lymphocytic leukemia: a decade later. Blood. 2011;118(13):3470-8.
17. Mraz, M.; Mraz, M.; Pospisilova, S.; Malinova, K.; Slapak, I.; Mayer, J. (). "MicroRNAs in chronic lymphocytic leukemia pathogenesis and disease subtypes". Leukemia & Lymphoma 2009;50(3):506–9.
18. Parker H, Rose-Zerilli MJ, Parker A, Chaplin T, Wade R, Gardiner A, et al. 13q deletion anatomy and disease progression in patients with chronic lymphocytic leukemia. Leukemia. 2011;25(3):489-97.
19. Falisi E, Novella E, Visco C, Guercini N, Maura F, Giaretta I, et al. B-cell receptor configuration and mutational analysis of patients with chronic lymphocytic leukaemia and trisomy 12 reveal recurrent molecular abnormalities. Hematol Oncol. 2013;Jul 17 [cited 2013 July 24, 2013] Available from http://www.ncbi.nlm.nih.gov/pubmed/23861036.
20. Willander K, Dutta RK, Ungerbäck J, Gunnarsson R, Juliusson G, Fredrikson M, et al. NOTCH1 mutations influence survival in chronic lymphocytic leukemia patients. BMC Cancer. 2013;13:274.
21. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008;111(12):5446-56.
22. Lobetti-Bodoni C, Bertoni F, Stussi G, Cavalli F, Zucca E. The changing paradigm of chronic lymphocytic leukemia management. Eur J Intern Med. 2013;24(5):401-10.
23. Rezvani AR, Maloney DG Rituximab resistance. Best Pract Res Clin Haematol. 2011;24(2):203-16.
24. Kolibaba KS, Sterchele JA, Joshi AD, Forsyth M, Alwon E, Beygi H, Kennealey GT. Demographics, treatment patterns, safety, and real-world effectiveness in patients aged 70 years and over with chronic lymphocytic leukemia receiving bendamustine with or without rituximab: a retrospective study. Ther Adv Hematol. 2013;4(3):157-71.
25. Te Raa GD, Malcikova J, Pospisilova S, Trbusek M, Mraz M, Garff-Tavernier ML, et al. Overview of available p53 function tests in relation to TP53 and ATM gene alterations and chemoresistance in chronic lymphocytic leukemia. European Research Initiative on CLL (ERIC). Leuk Lymphoma. 2013;54(8):1849-53.
26. Hulkkonen J, Vilpo L, Hurme M, Vilpo J. Surface antigen expression in chronic lymphocyte leukemia: clustering analysis, interrelationship and effects of chromosomal abnormalities. Leukemia. 2002;16(2):178-85.
27. http://www.cancer.gov/cancertopics/pdq/treatment/CLL/healthprofessional/ page2#Section_21. Accessed July 12, 2013.