Mature B- and T-Cell Lymphoproliferative Neoplasms

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[22.1] Chronic Lymphoproliferative Neoplasms
[22.2] Chronic Lymphocytic Leukemia
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Lymphoid neoplasms are broadly categorized into precursor lymphoid neoplasms and mature B-cell, T-cell, or natural killer (NK) cell neoplasms [Swerdlow 2008]. For practical purposes, mature B-, T-/NK-neoplasms can be further segregated into disorders likely to exhibit primary manifestations in blood and bone marrow (ie, chronic leukemias) compared with mature neoplasms that predominate in extramedullary sites but may involve blood or bone marrow more typically as a secondary event (B-, T-/NK- lymphomas). Furthermore, some mature B-cell/plasma cell disorders exhibit dominant immunosecretory manifestations. To aid the diagnostician in a logical systematic approach, the mature lymphoid/plasma cell neoplasms are separated into 4 chapters in this text:

1. chronic B- and T-cell leukemias, by convention termed chronic lymphoproliferative disorders/neoplasms (CLPD/CLPN), are the focus of this chapter
2. myeloma and other immunosecretory disorders are comprehensively reviewed in Chapter 23
3. bone marrow involvement by B- and T-cell lymphomas is the focus of Chapter 24
4. indolent and aggressive neoplasms of true NK cells are the focus of Chapter 26

### 22.1 Chronic Lymphoproliferative Neoplasms

Chronic lymphoproliferative neoplasms represent clonal proliferations of morphologically and immunophenotypically mature B or T cells characterized by a low proliferation rate and prolonged cell survival. **22.1** lists the specific subtypes of CLPNs, which are the focus of this chapter, based on the 2008 World Health Organization (WHO) criteria and definitions [Swerdlow 2008]. A systematic approach to CLPN generally begins with a morphologic review of blood smears **22.2**. Key decision-making steps in blood smear review include monotony vs heterogeneity of the lymphoid cells, absolute lymphocyte count, nuclear features such as chromatin condensation, and nuclear contours, as well as the features of the cytoplasm. It is also important to assess all other lineages for qualitative and quantitative features. For many of the CLPNs discussed in this chapter, the index of suspicion for an overt neoplastic disorder can be very high based on just morphologic and CBC data. However, multicolor flow cytometric immunophenotyping is the “mainstay” for establishing stage of maturation for B- and T-cell disorders and clonality for B-CLPN. Molecular assessment for T-cell receptor gene rearrangement is the gold standard for establishing T-cell clonality.

### 22.1 Chronic Lymphoproliferative Neoplasms with Characteristic Involvement of Blood and Bone Marrow at Presentation

<table>
<thead>
<tr>
<th>Mature B-Cell Neoplasms</th>
<th>Mature T-Cell Neoplasms*</th>
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<tbody>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>T-cell large granular lymphocytic leukemia</td>
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<tr>
<td>B-cell prolymphocytic leukemia</td>
<td>T-cell prolymphocytic leukemia</td>
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<td>Hairy cell leukemia</td>
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<td>Splenic zone lymphoma</td>
<td>Adult T-cell leukemia</td>
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<td>Splenic B-cell leukemia including hairy cell leukemia-variant</td>
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*Chronic lymphoproliferative disorders of natural killer (NK) cells and aggressive NK cell leukemias are the focus of Chapter 26

### 22.2 Approach to B- and T-Cell Chronic Lymphoproliferative Neoplasms (CLPN)

**For Diagnosis**

<table>
<thead>
<tr>
<th>Morphologic review of blood smear with CBC data</th>
<th>Determine stage of maturation based on nuclear features and likelihood of neoplastic vs non-neoplastic process</th>
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<tr>
<td>Immunophenotyping</td>
<td>Delineate full IP profile of lymphoid cells including patterns and intensity of surface/cytoplasmic antigen expression to confirm lineage and stage of maturation; Assess for neoplasm-specific markers</td>
</tr>
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<td>Genotyping</td>
<td>Assess for recurrent cytogenetic aberrations linked to specific CLPN subtypes; Molecular assessment for B- or T-cell clonality may be necessary for diagnosis of some CLPN</td>
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**For Prognosis**

<table>
<thead>
<tr>
<th>Staging</th>
<th>Largely based on clinical assessment, but cytopenias in blood are component of both Rai and Binet staging systems for CLL</th>
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<tr>
<td>Delineating other prognostic factors (major focus in CLL, not as relevant in other CLPN)</td>
<td>Major area of emphasis in CLL; CD38 and ZAP-70 assessment by IP in CLL; Karyotypic assessment by conventional cytogenetic/FISH in CLL and other B-, T-CLPN</td>
</tr>
<tr>
<td>Somatic mutation status by molecular assessment for CLL</td>
<td>Other specialized molecular/genetic testing may be useful for prognostication or in defining pathophysiologic mechanisms of leukemogenesis</td>
</tr>
<tr>
<td>Numerous other serum and molecular/genetic factors linked to prognosis (most notably in CLL)</td>
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</tbody>
</table>

CLL = chronic lymphocytic leukemia; FISH = fluorescence in situ hybridization
In addition, recurrent cytogenetic abnormalities may provide support for the diagnosis of some B- and T-CLPNs [22.2.9]. Although a final diagnosis has been the historical endpoint of the pathologic evaluation of neoplasms, the detection and delineation of numerous prognostic factors has become an area of much greater emphasis in CLPN, especially chronic lymphocytic leukemia (CLL).

Because flow cytometric immunophenotyping is readily available in most clinical practice settings, the discussions in this chapter will be segregated by B- or T-cell lineage, with the greatest emphasis on the most frequent CLPNs. Consequently, B-CLPN will be presented first, followed by T-CLPN.

### [22.2] Chronic Lymphocytic Leukemia

#### [22.2.1] General Features

Although it has a worldwide distribution, CLL is much more frequent in Western countries, where it accounts for up to 30% of all leukemias [Montserrat 2008]. This disease characteristically affects middle-aged to elderly patients, especially men, with a median age of 70 years. CLL can occur in younger patients (≤55 years); a more aggressive disease course has been noted in these patients [Mauro 1999]. The incidence of CLL increases dramatically with age and may exceed 50 in 100,000 men >80 years of age [Dores 2007]. Most patients are asymptomatic, and the disease is detected by complete blood count (CBC) assessment. Symptomatic patients may manifest with lymphadenopathy, various autoimmune disorders such as hemolytic anemia or immune-mediated thrombocytopenia, or, less commonly, fatigue, fever, and weight loss [Dearden 2008, Fuller 2008, Montserrat 2008, Morton 2008, Visco 2008].

Some insights into the pathogenesis of CLL have been delineated from both the assessment of familial kindreds and sophisticated evaluations of CLL cells. Since CLL is the most common familial leukemia, both genetic linkage studies and \( IGHV \) mutation analyses of kindreds have helped to define genomic linkages and an association of gene usage patterns in familial CLL [Aoun 2007, Brown 2008, Crowther-Swanepoel 2008, Ng 2007]. Even the link between CLL and monoclonal B-lymphocytosis has been solidified by family studies (see [22.2.9] "Differential Diagnosis," p 485) [Fuller 2008]. Recent studies have provided numerous insights into the pathogenesis of CLL, but a clear-cut linear delineation of the precise, sequential pathogenesis of CLL has not been forthcoming. Factors at play in the pathogenesis of CLL include chronic antigenic stimulation, the B-cell surface receptor molecule, the bone marrow microenvironment, and numerous genomic alterations, especially microRNA mutations sidebar 22.1. [Caligaris-Cappio 2008, Chiorazzi 2005, Linet 2007]

#### [22.2.2] Blood Findings

The diagnosis of CLL requires the integration of absolute monoclonal B lymphocyte count, lymphocyte morphology, and the immunophenotypic profile of the neoplastic cells [22.3]. An unexplained, sustained absolute monoclonal B lymphocytosis of \( >5.0 \times 10^9/\mu\text{L} \) (≥5.0 × 10^9/L) or more for 3 months is required [Hallek 2008, Muller-Hermelink 2008]. Studies subsequent to the 2008 WHO classification indicate that an absolute monoclonal B lymphocytosis of \( 1.1 \times 10^9/\mu\text{L} \) (11 × 10^9/L) is highly predictive of outcome (see Prognostic Factors section). Consequently, requirements for diagnosis may differ from outcome predictors. Similarly, the diagnostican needs to be aware that criteria used to distinguish overt low-stage B-CLL from monoclonal B lymphocytosis may be revised based on these recent studies (see [22.2.9] "Differential Diagnosis," p 485) [Martí 2009, Rossi 2009, Shanafelt 2009].

### [22.3] Prototypic Features of Chronic Lymphocytic Leukemia (CLL)

#### Blood

- Absolute monoclonal B lymphocyte count ≥5.0 × 10^9/μL (≥5.0 × 10^9/L) with CLL immunophenotype for 3 months*
- Mature, monotonous lymphocytes with round nuclei, condensed chromatin, inconspicuous nucleoli, and generally scant cytoplasm

#### Bone marrow

- Mature lymphocytosis generally >30% of total cells
- Focal nonparatrabeclular or diffuse infiltrates on core biopsy sections
- Only rarely see proliferation foci on core biopsy; true germinal centers very rarely noted

#### Immunophenotype (IP)

- CD5+ monoclonal light chain-restricted B cell with dim CD20, dim CD22, CD23, and weak surface immunoglobulin
- Antigens typically absent: CD10, FMC7, and CD79b
- \( IGHV \) mutation status (molecular)
- MicroRNA gene deletions or gene overexpression (not yet utilized in routine clinical practice) (see sidebar 22.1)

#### Key prognostic factors (assessed at diagnosis)

- Clinical stage (Rai, Binet)
- Absolute monoclonal B lymphocyte count of \( ≥11 \times 10^9/\mu\text{L} \) (≥11 × 10^9/L)
- CD38 expression (IP)
- ZAP-70 expression (IP)
- Karyotype (FISH)

### References

Recent advances in genome biology have highlighted the central role of genetic abnormalities and their consequences for protein expression or function. The recently discovered and incompletely understood microRNA (miRNA) world, sometimes termed the miRNAome, is yet another level at which cellular homeostasis in these neoplasms is disturbed. Just as genetic abnormalities provide an opportunity for diagnostic and prognostic investigations, so alterations at the miRNA level are proving to be a promising determinant of neoplastic pathogenesis and behavior. Among hematopoietic malignancies, CLL is the most extensively studied at this level.

As described in sidebar 1.1 (p.4), miRNAs are short RNA molecules, coded for by specific regions of the genome, which are not translated into a protein product, as is the fate of traditional messenger RNA (mRNA). Instead, miRNAs are processed and incorporated into the RNA-induced silencing complex (RISC), whereupon they suppress the translation of other target miRNAs. The nucleotide sequence of the miRNA is complementary to that of portions of the target mRNA, providing specificity to the translational repression. Thus far, hundreds of miRNAs have been identified in human cells, and their presence affords an additional level of control over gene expression beyond that achieved through strictly genetic and epigenetic means. Greater than 30% of the human genome may be under translational regulation by miRNA [Schickel 2008]. It is theorized that miRNA may have originated as a primitive cellular defense against RNA viruses or transposons [Zhang 2007].

Theorized that miRNA may have originated as a primitive cellular defense against RNA viruses or transposons [Zhang 2007].

One of the most commonly affected chromosomal regions in CLL is 13q14.3, isolated deletions of which confer a relatively good prognosis. The frequency and consistency with which deletion of 13q14.3 arises in CLL indicates that a tumor suppressor gene is likely present at this location, but extensive evaluation of the known genes in the minimally deleted region did not reveal a convincing candidate [Aqeilan 2010]. However, after the discovery and early characterization of miRNAs, investigators identified 22 sequences encoding miRNAs within the critical region [Cali 2002, Nicolaou 2007]. Interestingly, these miRNA sequences were located within an intron of another gene, DLEU2, which had been previously considered and largely excluded as a candidate tumor suppressor at this locus [Nicolaou 2007]. The specific miRNAs encoded, miR-15a and miR-16-1, were found to be highly expressed in CD5+ lymphocytes, and to be downregulated or deleted in 70% of CLL cases [Cali 2002; miR-15a and miR-16-1 both target for interference and/or destruction mRNA transcribed from the anti-apoptotic gene BCL2, providing a plausible mechanism by which 13q14.3 deletion could confer a proliferative advantage [Cimmino 2005].

Recently, yet another common chromosomal abnormality in CLL has been functionally linked to miRNAs. The well-known tumor suppressor TP53, which is deleted in cases of CLL with loss at 17p, positively regulates the transcription of miR-34a [Cali 2009]. Low levels of miR-34a in CLL are associated with resistance to chemotherapy and to apoptosis, suggesting the participation of this miRNA in generating the adverse prognostic phenotype associated with TP53 deletion in CLL [Zenz 2009].

While cytogenetic abnormalities are of great importance in CLL, other cellular characteristics, including ZAP70 expression and IGHV somatic hypermutation status, are known to be prognostically significant. An initial study examining global miRNA expression in subsets of CLL delineated by such prognostic markers showed that favorable and unfavorable prognostic groups maintain largely distinct miRNA expression patterns [Cali 2008]. This and similar studies have consistently demonstrated downregulation of miR-223 and members of the miR-29 family in CLL [Cali 2008, Falci 2007, Marton 2008], with low levels specifically associated with disease progression, poor prognostic factors, and reduced treatment-free and overall survival [Stamatopoulos 2009].

Given that we are only beginning to unravel the processes by which miRNAs affect cellular function, it is highly likely that the success of these initial investigations will be replicated in the future, yielding further dramatic insight into the pathogenesis of CLL and of hematopoietic malignancies in general. One might even hazard a prediction that miRNA-based data could some day be incorporated into diagnostic classification schemes, much as chromosomal abnormalities and alterations involving protein-coding genes have assumed a central place in the current 2008 WHO system.

In general, hematopoietic lineages are preserved in patients with CLL, especially in asymptomatic patients. If cytopenias are detected, this can reflect either a more advanced stage of the disease with bone marrow effacement or an immune-mediated destruction of cells secondary to autoantibody production [Montserrat 2008]. Likewise, rare cases of anemia secondary to red cell aplasia have been described in CLL patients, a presumed consequence of the T-cell aberrations that are well-described in this disorder [Lanasa 2009].

In CLL, a monotonous population of mature lymphocytes is generally identified in the blood, although some heterogeneity is noted occasionally i22.1, i22.2, i22.3. These cells have round nuclei and highly condensed nuclear chromatin with separation of chromatin and parachromatin, creating a blocky pattern i22.4. Cytoplasm is generally scant, but may be more moderate in some cases that exhibit otherwise typical morphologic features of CLL i22.5. In rare cases, the leukemic
cells have cytoplasmic vacuoles or crystals derived from monotypic immunoglobulin \textsuperscript{22.6, 22.7} [Peters 1984, Ralfkiaer 1982].

In addition to the monotonous lymphocytes that typify B-CLL, a range of nuclear aberrations may be noted in cases of otherwise straightforward CLL. For example, a small portion of these lymphoid cells (usually <5\%) may exhibit prominent nuclear clefting reminiscent of that seen in mantle cell and follicular lymphomas. Also, the proportion of lymphocytes with prominent nucleoli (prolymphocytes), although generally low and stable over time, may sometimes be increased at presentation \textsuperscript{22.8}. A progressively increasing proportion of prolymphocytes on sequential specimens may indicate disease transformation (see Transformations section).

The designation “atypical” CLL has been applied under several circumstances. The more common usage relates to cases of CLL in which the morphologic features differ from classic CLL, while other parameters (clinical, CBC, and immunophenotypic profile) are characteristic of CLL. In these cases of so-called atypical CLL, the circulating lymphocytes are more heterogeneous, and both nuclear irregularity and nucleolated lymphocytes are more prominent than prototypic CLL \textsuperscript{22.9, 22.10, 22.11}. Associations between morphologic atypia and genotype have been described (see Cytogenetics section). The other circumstance in which atypical CLL has been used relates to cases in which the immunophenotypic profile differs somewhat from prototypic CLL (see Immunophenotypic Analysis section). Caution should be exercised in using the term atypical CLL in that differential diagnostic considerations must be thoroughly assessed and excluded.

\textsuperscript{[22.2.3] Bone Marrow Findings

Bone marrow examination should be considered in patients with CLL to determine both the pattern and extent of the leukemic infiltrates and to evaluate the residual hematopoietic cells. On bone marrow aspirate smears, lymphocytes generally account for >30\% of cells, and they exhibit cytologic features similar to those of circulating lymphocytes \textsuperscript{22.12, 22.13}. The evaluation of good quality bone marrow core biopsy sections is especially useful in determining pattern and extent of infiltration in CLL. CLL infiltrates are designated as nodular, interstitial, diffuse, or mixed, which is a combination of either nodular and interstitial or nodular and diffuse. Because of the scant cytoplasm and closely packed nuclei that typify CLL, these leukemic infiltrates are characteristically dark blue on low magnification of bone marrow biopsy sections.

Nodular infiltrates of CLL are characteristically nonparatrabecular in location and are typically composed of small lymphocytes with very closely packed nuclei exhibiting round nuclear contours and condensed chromatin. Little mitotic activity is evident \textsuperscript{22.14, 22.15, 22.16, 22.17} [Hsi 2009, Viswanatha 2010]. These nodules generally demonstrate infiltrative margins with
Blood smear depicts cytoplasmic vacuoles in a chronic lymphocytic leukemia cell. (Wright)

Blood smear illustrates prolymphocytes from 3 cases of chronic lymphocytic leukemia. Note the more dispersed chromatin with central nucleoli that characterizes these more immature cells. (Wright)

Blood smear illustrates numerous immunoglobulin crystals in the cytoplasm of chronic lymphocytic leukemia cells. (Wright)

This peripheral blood smear from a 57-year-old male with atypical chronic lymphocytic leukemia shows a greater heterogeneity in cell size and nuclear configurations. This patient had concurrent cytopenias. (Wright)

This peripheral blood smear from a patient with atypical chronic lymphocytic leukemia shows a fairly significant range in cell size. (Wright)

Large prolymphocytic cells are present in this peripheral blood smear from a patient with atypical chronic lymphocytic leukemia. (Wright)
percolation of small lymphocytes into the surrounding hemato-
poietic cells. Features that help to distinguish these neoplastic
nodules from benign lymphoid aggregates include the relatively
higher number of nodules, the infiltrative margins, the
monomorphic cellular composition, and the lack of a consistent
perivascular distribution in CLL (see Volume 1, Chapter 21
for a discussion of benign lymphoid aggregates). In addition,
immunoperoxidase staining can be used to document the
predominance of B cells coexpressing either CD5 or CD43 in
CLL, while T cells generally predominate in benign lymphoid
aggregates. Interstitial infiltates of CLL consist of an admixture
of CLL cells with residual fat and hematopoietic cells
The “mixed” designation usually refers to
an admixture of nodular and interstitial infiltates, while
complete effacement of both fat and hematopoietic cells
defines diffuse solid infiltates of CLL. Infiltates can also
occasionally involve bone marrow sinuses in conjunction with
other patterns. The cytologic features of both these
interstitial and densely packed diffuse cellular infiltates are
similar to those found in the nodular lesions.

Unlike the situation in an involved lymph node, prolif-
eration foci are only rarely identified in bone marrow core
biopsy sections, possibly a reflection of the small sample
size. Reports of non-neoplastic germinal centers in
core biopsy sections from CLL patients are even more rarely
encountered, at essentially the “case report” frequency

Occasional large transformed cells may be admixed
with the small lymphocytes in CLL infiltates within the
bone marrow. As long as the proportion of these larger
cells is low, there is no adverse impact on survival. In rare
cases, Reed-Sternberg-like cells exhibiting multinucleation
and huge nucleoli are admixed with otherwise typical CLL
infiltates. The significance of this finding

Bone marrow biopsy section illustrates nodular infiltates of chronic
lymphocytic leukemia that are primarily nonparatrabecular. (H&E)

Many focal nonparatrabecular nodules of chronic lymphocytic
leukemia are present on this bone marrow biopsy section. (H&E)
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i22.16 Vague proliferation foci are present in a large chronic lymphocytic leukemia nodule on this bone marrow biopsy section. (H&E)

i22.17 High magnification of nodular lymphoid aggregate in the bone marrow core biopsy section from a patient with chronic lymphocytic leukemia shows relative uniformity in cell size and little, if any, mitotic activity. (H&E)

i22.18 Immunohistochemical staining for CD20 on this bone marrow core biopsy section shows numerous B cells with weak to intermediate positivity (see i22.17). (immunoperoxidase for CD20)

i22.19 Immunohistochemical staining for CD3 on this lymphoid nodule from a patient with chronic lymphocytic leukemia shows only scattered T cells (see i22.17 and i22.18). (immunoperoxidase for CD3)

i22.20 This low magnification of a bone marrow core biopsy section from a patient with extensive diffuse interstitial infiltration of chronic lymphocytic leukemia shows normal bony trabeculae and relatively preserved fat. (H&E)

i22.21 On high magnification diffuse interstitial infiltrates of chronic lymphocytic leukemia show an admixture of the leukemia cells with preserved fat and occasional hematopoietic cells, most often megakaryocytes. (H&E)
is controversial, because some of these patients eventually develop overt Hodgkin lymphoma, while others do not. Likewise, some cases exhibit an immunophenotype of true Reed-Sternberg cells, while these multinucleated cells exhibit a straightforward B-cell phenotype in other cases [Kanzler 2000, Momose 1992, Williams 1991, Ohno 1998]. Finally, in patients with known, longstanding CLL, foci of transformation to large cell lymphoma may be evident on either aspirate smears or biopsy sections (see Transformations section).

Recent studies provide insight into the association of a diffuse pattern of CLl infiltration in bone marrow with progressive disease and an overall more aggressive disease course. Both ZAP-70 expression and unmutated IGHV genes are linked to a diffuse core biopsy infiltration pattern [Schade 2006, Zanotti 2007].

**Immunophenotypic Analysis**

Multicolor flow cytometric immunophenotyping is essential in establishing a diagnosis of CLl, in delineating specific prognostic factors (CD38 and ZAP-70 assessment), and in distinguishing CLl from other leukemic CLPN [Chiorazzi 2005, Hallek 2008, Morice 2008]. In addition to weak monotypic surface immunoglobulin, the classic immunophenotypic profile of CLl includes expression of CD19, weak CD20, weak CD22, weak CD11c, CD5, and CD23 antigens. Cases of CLl characteristically lack CD25, CD10, and FMC7, while CD79b expression is weak or absent [McCarron 2000, Morice 2008]. Variations from this prototypic immunophenotypic profile have been described including partial or absent CD23 expression, partial or absent CD5 expression, and bright CD20 expression [Morice 2008]. The designation “atypical CLl” is sometimes applied to cases with
“nonclassic” immunophenotype [Frater 2001]. Similar to cases of CLL with atypical morphology, cases of CLL with atypical immunophenotypic features have been linked to distinctive genetic and clinical features (see Cytogenetics section) [Frater 2001, Schlette 2003, Tan 2008]. When cell suspensions for flow cytometry are not available, paraffin immunoperoxidase techniques can be used to assess CD20, cyclin D1, CD5, CD43, CD10, and CD23, further facilitating the distinction between CLL and other B-CLPN [22.24, 22.25, 22.26, 22.27] (see [22.19] “Differential Diagnosis,” p 485).

### 22.4 CD38 Expression in CLL

Surface ADP-ribosyl cyclase adhesion molecule that supports B-cell interactions and differentiation

Induces proliferation and activation, and increases survival of CLL cells

Key element, in conjunction with ZAP-70, in coreceptor pathway that may sustain B-cell receptor signals

Surface expression on ≥30% of CLL population independent prognostic factor

Functionally linked with ZAP-70 and confers enhanced migratory potential on CLL cells

Level of expression may vary during disease course and by tissue site

CLL = chronic lymphocytic leukemia; ZAP-70 = zeta-chain-associated protein kinase

22.5 ZAP-70 Expression in CLL

Zeta-associated protein of 70 kDa

Cytoplasmic tyrosine kinase essential for T-cell receptor signal transduction

Expressed in normal and neoplastic B cells at different maturation stages

Originally identified by gene expression profiling studies comparing CLL with and without IGHV somatic mutations

Although linked to somatic mutation status some investigators report that ZAP-70 expression has higher relative value in predicting outcome compared with CD38 expression and mutation status

May be assessed by flow cytometry and immunohistochemistry, but highly problematic in many laboratories

CLL = chronic lymphocytic leukemia; ZAP-70 = zeta-chain-associated protein kinase


22.6 Cytogenetic Features of CLL

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>% of Cases</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Normal</td>
<td>20%-30%</td>
<td>Linked to intermediate outcome</td>
</tr>
<tr>
<td>del(13q14)</td>
<td>&gt;40%</td>
<td>Sole abnormality in 50% of untreated patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linked to better outcome when sole abnormality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linked to mutated IGHV, absence of CD38, typical morphology</td>
</tr>
<tr>
<td>trisomy 12</td>
<td>7%-25%</td>
<td>Linked to intermediate outcome, CD38 expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linked to atypical morphology, bright CD20 expression, and high rate of response to rituximab</td>
</tr>
<tr>
<td>del(11q)</td>
<td>3%-15%</td>
<td>Linked to unmutated IGHV, adverse outcome, ATM gene deletions/alterations</td>
</tr>
<tr>
<td>del(17p)</td>
<td>5%-12%</td>
<td>TP53 mutations/inactivation may be independent of del(17p) and linked to low expression of miR-34a</td>
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<td></td>
<td></td>
<td>Linked to rapid disease progression, adverse outcome</td>
</tr>
<tr>
<td>14q32 translocations</td>
<td>4%-10%</td>
<td>Multiple translocation partners</td>
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<tr>
<td></td>
<td></td>
<td>Poor outcome, CD38 expression</td>
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<tr>
<td>Complex karyotypes</td>
<td>10%-40%</td>
<td>Linked to unmutated IGHV, CD38 expression</td>
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<tr>
<td></td>
<td></td>
<td>Linked to short telomeres, short survival, and TP53 mutations</td>
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CLL = chronic lymphocytic leukemia


Cytogenetic and Molecular Features


Somatic Mutations of IGHV Genes

Assessment for somatic mutations of IGHV genes in CLL is the cornerstone in separating CLL into 2 biologic and prognostic groups. Cases of CLL with unmutated (germline) IGHV genes show a characteristic profile consisting of CD38 and ZAP-70 expression, adverse karyotype, reduced time to therapy, and overall more aggressive disease course [Chiorazzi 2005, Montserrat 2008, Morabito 2009, Murray 2008, Rassenti 2008]. In contrast, the biologically indolent subtype of CLL is characterized by somatic hypermutations, absence of CD38 and ZAP-70, and good risk karyotype. Recent evidence suggests that both of these biologic CLL subtypes are derived from an antigen-experienced B cell but differ based on...
the extent of somatic mutation [Chiorazzi 2005]. Consequently, the designation germline/unmutated, is not entirely correct, but by convention is used to delineate the biologically aggressive CLL subtype.

[217] Integration of Primary Prognostic Factors

In patients with CLL in whom all of these immunophenotypic and genetic prognostic factors are concordant and favorable, the median survival time may exceed 25 years [Shanafelt 2004]. In contrast, in patients with CLL in whom all of these prognostic factors are concordant and unfavorable, the median survival time is ≤8 years. Survival time for patients whose CLL cells show discordant CD38 and ZAP-70 is intermediate [Schroers 2005, Shanafelt 2004].

[2218] Other Prognostic Factors

In addition to traditional Rai and Binet staging and the previously discussed biologic markers (CD38, ZAP-70 expression, somatic mutation status, and karyotype), other prognostic factors have been described in CLL [Monzo 2008, Van Bockstaele 2009]. These range from routine hematologic factors such as lymphocyte doubling time to various serum markers (soluble CD23 doubling time, LDH, β-2 microglobulin) to esoteric markers such as microRNA signature profile (see sidebar 22.1, p 477), thrombopoietin levels, telomere length, and the expression of various genes (TCL1, MCL1, MDRI, and antiapoptotic genes) [Agrawal 2008, Delgado 2009, Koller 2006, Menken 2008, Moreno 2008, Pepper 2008, Ricca 2007, Weinberg 2007, Wierda 2007, Zen 2009]. Of note, initial therapeutic modalities affect the prognostic significance of many of these biologic factors [Lin 2009, Wierda 2009].

[2219] Differential Diagnosis

When an elderly patient presents with a markedly elevated monotonous, mature lymphocytosis, the diagnosis of CLL is generally not difficult. However, the diagnosis of CLL is much more challenging in patients in whom the absolute lymphocyte count is only minimally elevated or in cases in which the morphologic and clinical features are not those associated with classic CLL. Disorders that can show morphologic overlap with CLL include reactive lymphocytoses, monoclonal B lymphocytosis, B-cell prolymphocytic leukemia (B-PLL), hairy cell leukemia (HCL), splenic marginal zone lymphoma, and some other peripheralized non-Hodgkin lymphomas [22.7] [Delage 2001, Himmelmann 2001, Karandikar 2002, Landgren 2009, Ravot 2008, Troussard 1996]. Because most reactive lymphocytoses are composed of morphologically heterogeneous T cells, their distinction from CLL is usually possible, often by morphologic review of the blood smears and clinical correlation. Both the pronounced morphologic heterogeneity and the association with viral syndromes in reactive transient, reactive lymphocytoses is generally sufficient for their distinction from clonal disorders; immunophenotyping studies are not generally required. However, there are rare reports of unexplained, sustained, mature lymphocytes in young women (see Volume 1, Chapter 21) [Delage 2001, Himmelmann 2001, Troussard 1996]. The polyclonal B-cell surface antigen profile of these processes can be used to exclude CLL from consideration.

For clonal B-cell disorders ranging from monoclonal B lymphocytosis to peripheralized lymphomas, the distinction from CLL may be more challenging, requiring the integration of clinical, morphologic, immunophenotypic, genetic, and outcome information. Several large studies of normal individuals confirm that occult B-cell clones, termed monoclonal B lymphocytosis, are common in blood, especially in older patients [Landgren 2009, Nieto 2009, Ravot 2008]. These occult clones often, but not always, exhibit a CLL immunophenotypic profile and are typically detected in individuals with a normal absolute lymphocyte count. Although much more frequent than overt CLL, monoclonal B lymphocytosis is a likely precursor lesion because it was detected retrospectively in 98% of individuals who developed overt CLL in a cancer screening clinical trial [Landgren 2009]. Recent studies suggest that the criteria for distinguishing monoclonal B lymphocytosis from overt low-stage CLL may need refinement, since the level of absolute monoclonal B lymphocyte count which is most significantly correlated with outcome is 11 × 103/μL (11 × 109/L) [Marti 2009, Shanafelt 2009]. In addition, patients with small lymphocytic lymphoma may manifest minor blood involvement that may mimic monoclonal B lymphocytosis. The diagnostician must be aware of these potential pitfalls that may lead to either overdiagnosis or underdiagnosis of small monoclonal B-cell populations in blood.

The spectrum of features useful in distinguishing CLL from other overt B-cell neoplasms is delineated in various ways on 22.8, 22.9, 22.10, 22.11. Again, a systematic multiparameter approach is essential. The diagnostician must be aware, however, that some cases may be equivocal, even after extensive assessment. In these situations, a diagnosis of CLPN, not otherwise specified (NOS), should be considered. Indeed, the diagnostic challenge of CD5+/CD10– leukemias has been well described, and lymphoid leukemias with a typical mantle cell lymphoma (MCL) phenotype may include cases of CLL by genetic assessment [Goldman 2008, Ho 2009].

[221.0] Transformations

The development of a second hematolymphoid neoplasm in a patient with longstanding CLL represents a diagnostic challenge: is this neoplasm a transformation of the CLL or a therapy-related secondary disorder? For example, the alkylating agent therapy used to treat some patients with CLL can induce either secondary myelodysplasia or acute myeloid leukemia (see Volume 1, Chapters 16 and 18) [22.8, 22.9]. In contrast, many
### 22.7 Differential Diagnosis of CLL

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Key Distinguishing Feature in Clinical/Blood Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive lymphocytosis*</td>
<td>T cells predominate</td>
</tr>
<tr>
<td></td>
<td>Usually virally induced; transient</td>
</tr>
<tr>
<td></td>
<td>Prominent morphologic spectrum</td>
</tr>
<tr>
<td>Transient stress lymphocytosis*</td>
<td>Modest absolute lymphocytosis after severe stress</td>
</tr>
<tr>
<td></td>
<td>Transient increase in helper and suppressor T cells as well as B and NK cells</td>
</tr>
<tr>
<td>Polyclonal B lymphocytosis*</td>
<td>Familial link; HLA link</td>
</tr>
<tr>
<td></td>
<td>Young females, heavy smokers</td>
</tr>
<tr>
<td></td>
<td>Binucleated lymphocytes; sustained</td>
</tr>
<tr>
<td>Monoclonal B lymphocytosis</td>
<td>CLL-like immunophenotype</td>
</tr>
<tr>
<td></td>
<td>Detected in up to 12% of normal individuals, especially elderly</td>
</tr>
<tr>
<td></td>
<td>Increased frequency of detection with aging (100 times more frequent than overt CLL)</td>
</tr>
<tr>
<td></td>
<td>Absolute lymphocyte count usually normal</td>
</tr>
<tr>
<td></td>
<td>Likely precursor to overt CLL in some, but not all, cases</td>
</tr>
<tr>
<td></td>
<td>Identified retrospectively in 98% of patients who developed overt CLL in a population-based cancer screening clinical trial</td>
</tr>
<tr>
<td></td>
<td>Risk of progression to treatment requirement linked to trisomy 12 or del(17p13)</td>
</tr>
<tr>
<td>B-cell prolymphocytic leukemia</td>
<td>WBC strikingly increased</td>
</tr>
<tr>
<td></td>
<td>Prolymphocytes predominate</td>
</tr>
<tr>
<td></td>
<td>Lacks both IP and genetic features of CLL</td>
</tr>
<tr>
<td></td>
<td>Usually associated with marked hepatosplenomegaly</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>Cytopenias predominate, often pancytopenia</td>
</tr>
<tr>
<td></td>
<td>Neutropenia, pronounced monocytopenia</td>
</tr>
<tr>
<td></td>
<td>Hairy cells usually rare in blood</td>
</tr>
<tr>
<td></td>
<td>Very distinctive morphology of hairy cells</td>
</tr>
<tr>
<td></td>
<td>Very distinctive IP profile</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>Mild leukocytosis with spectrum with plasmacytoid cells; bipolar projections</td>
</tr>
<tr>
<td></td>
<td>Lacks both IP and genetic features of CLL</td>
</tr>
<tr>
<td></td>
<td>Splenomegaly typical</td>
</tr>
<tr>
<td>Other peripheralized B-cell lymphomas</td>
<td>Often have clinical history of NHL in extramedullary sites</td>
</tr>
<tr>
<td></td>
<td>May exhibit de novo leukemic manifestations (especially mantle cell lymphomas)</td>
</tr>
<tr>
<td></td>
<td>Nuclear irregularities of lymphoid cells common</td>
</tr>
<tr>
<td></td>
<td>Lack both IP and genetic features of CLL</td>
</tr>
</tbody>
</table>

*See Volume 1, Chapter 21 for more details

CLL = chronic lymphocytic leukemia; IP = immunophenotype; NHL = non-Hodgkin lymphoma

### 22.9 Defining Immunophenotypic Features* in Chronic B-Cell Neoplasms

<table>
<thead>
<tr>
<th></th>
<th>CLL</th>
<th>B-PLL</th>
<th>HCL</th>
<th>MCL</th>
<th>SMZL</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD20</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dim</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bright</td>
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<tr>
<td><strong>CD22</strong></td>
<td></td>
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<tr>
<td>Dim/absent</td>
<td>+</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bright</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>CD23</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Light chain</strong></td>
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</tr>
<tr>
<td>Dim</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bright</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>CD79b</strong></td>
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<tr>
<td></td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>CD11c</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Weak/moderate</td>
<td>+</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Bright</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>CD10</strong></td>
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<tr>
<td></td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td><strong>CD25</strong></td>
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<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>CD5</strong></td>
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<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<tr>
<td><strong>FMC7</strong></td>
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<tr>
<td></td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Only characteristic immunophenotypic features given; many exceptions reported
†Grading system:  +   = Typical feature of the disease
0   = Not a defining feature of disease; may be present but does not have diagnostic specificity
– = Feature which does not support diagnosis of disease (can be used to refute diagnosis)

CLL = chronic lymphocytic leukemia; B-PLL = B-cell prolymphocytic leukemia; HCL = hairy cell leukemia; MCL = mantle cell lymphoma; SMZL = splenic marginal zone lymphoma; FL = follicular lymphoma

### 22.10 Pattern and Morphology of Bone Marrow Involvement in B-Chronic Lymphoproliferative Neoplasms (B-CLPN)*

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Aspirate Morphology</th>
<th>Core Biopsy Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL/SLL</td>
<td>Monotonous small, round lymphocytes with scant cytoplasm</td>
<td>Focal nonparatrabecular infiltrates predominate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other patterns include diffuse interstitial or diffuse solid infiltrates</td>
</tr>
<tr>
<td>B-PLL</td>
<td>Intermediately sized lymphocytes exhibiting round nuclei with relatively condensed nuclear chromatin and prominent central nucleoli</td>
<td>Usually diffuse</td>
</tr>
<tr>
<td>HCL</td>
<td>Distinctive cell with reniform nuclei, spongy “checkerboard” nuclear chromatin, and moderate to abundant amounts of slightly basophilic cytoplasm exhibiting circumferential shaggy contours</td>
<td>Diffuse interstitial and sinusoidal infiltrates characteristic; can be very subtle and best appreciated by immunohistochemical assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discrete, nodular lesions distinctly uncommon</td>
</tr>
<tr>
<td>MCL</td>
<td>Small to intermediately sized lymphocytes with variably condensed chromatin and irregular nuclear contours; a variable number of cells may exhibit either prolymphocytic or blastic features</td>
<td>Usually focal nonparatrabecular and/or paratrabecular infiltrates evident, although interstitial and diffuse lesions also described</td>
</tr>
<tr>
<td>FL</td>
<td>Variable, but small cleaved lymphoid cells typically predominate</td>
<td>Focal paratrabecular lesions predominate</td>
</tr>
<tr>
<td>MZL (including splenic MZL)</td>
<td>Variable with admixture of plasmacytoid forms Some cells may exhibit shaggy cytoplasmic contours that tend to be bipolar</td>
<td>Discrete nodular lesions very characteristic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be associated with sinusoidal infiltrates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May see “naked” germinal centers</td>
</tr>
</tbody>
</table>

*See Chapter 24 for more details

CLL/SLL = chronic lymphocytic leukemia/small lymphocytic leukemia; B-PLL = B-cell prolymphocytic leukemia; HCL = hairy cell leukemia; MCL = mantle cell lymphoma; FL = follicular lymphoma; MZL = marginal zone lymphoma including splenic MZL

Mature B- and T-Cell Lymphoproliferative Neoplasms

subsequent lymphoid lesions that develop in patients with CLL likely represent transformations of the original disease [22.12].

The most common transformation, occurring in 5%-15% of cases of CLL, is the development of increased numbers of prolymphocytes, so-called prolymphocytic transformation of CLL. This transformation may be associated with progressive disease, loss of responsiveness to chemotherapeutic agents, and decreased survival time [22.30, 22.31, 22.32, 22.33]. The increasing proportion of prolymphocytes with prominent central nucleoli may be linked to clonal progression on cytogenetic studies and to changes in the immunophenotypic profile [Kroft 2001, Schlette 2003, Viswanatha 2010]. If increased prolymphocytes are a feature at the time of initial presentation, disease course cannot be predicted, in that some cases demonstrate a prolonged stable disease course, while others exhibit disease progression. In contrast, the development of a progressively rising prolymphocyte count in patients with established CLL is more compatible with a prolymphocytoid transformation. These prolymphocytic cells retain many of the immunophenotypic features of the original CLL, though both surface immunoglobulin and CD22 expression may be brighter, while CD23 and CD5 coexpression may diminish [22.3].

The development of a large cell lymphoma in a patient with CLL, so-called Richter syndrome, is a more dramatic event that occurs in 2%-12% of patients with established CLL [Aydin 2008, Mao 2007, Richter 1928, Rossi 2008, Swords 2007]. This phenomenon is relatively more common in younger patients with CLL (≤55 years) [Mastro 1999]. These patients experience
the abrupt onset of severe symptoms, often related to large, extramedullary lymphomatous mass lesions composed of pleomorphic, large immunoblastic cells. Foci of large cell transformation may be evident in the bone marrow of some patients, but in most patients the bone marrow is uninvolved at the time of extramedullary large cell

**22.12 Types of Transformation of CLL**

<table>
<thead>
<tr>
<th>Type of Transformation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolymphocytoid</td>
<td>Occurs in 5%-15% of cases of CLL&lt;br&gt;Gradual increase in prolymphocytes in blood and bone marrow&lt;br&gt;May be associated with progressive symptoms, refractoriness to therapy&lt;br&gt;Phenotype similar to original CLL but minor changes described</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma or rarely Hodgkin-like transformation (Richter syndrome)</td>
<td>Occurs in 2%-15% of CLL patients (15% at 10 years)&lt;br&gt;Abrupt onset of severe symptoms&lt;br&gt;Large mass lesions develop that may be extranodal&lt;br&gt;Often immunoblastic and pleomorphic; rarely Hodgkin-like&lt;br&gt;Very aggressive disease with median survival &lt;1 year&lt;br&gt;Clonal association with underlying CLL established in most cases (about 80%)</td>
</tr>
<tr>
<td>Other putative transformations</td>
<td>Rare reports of acute lymphoblastic leukemia, Burkitt lymphoma, myeloma, hairy cell leukemia, and even aggressive T-cell disorders have been described as possible “transformations” of CLL&lt;br&gt;Supporting data marginal; may be secondary neoplasms</td>
</tr>
</tbody>
</table>

CLL = chronic lymphocytic leukemia

lymphomatous transformation. Rarely, both blood and bone marrow involvement by large cell lymphoma may be evident in some cases the large cell transformation has a Hodgkin lymphoma appearance. Most recent evidence suggests a clonal relationship between the large cell lymphoma of Richter syndrome and CLL, but some cases, including Hodgkin-like Richter syndrome, represent secondary immunodeficiency-associated disorders, often Epstein-Barr virus (EBV) induced. A link to fludarabine therapy and subsequent Hodgkin lymphoma has been suggested in one study.

Chronic lymphocytic leukemia factors linked to the subsequent development of Richter syndrome include unmutated IGHV, lymph node size, and absence of del(13q14) [Rossi 2008]. Other studies propose an association of Richter syndrome with CD38 gene polymorphisms, high levels of activation-induced cytidine deaminase, or aberrant somatic mutations [Aydin 2008, Reiniger 2006].

Other putative types of transformation of CLL are rare and include the development of either a B lymphoblastic leukemia or plasmablastic (myelomatous) blood and bone marrow picture, while both nodal paraimmunoblastic or Burkitt lymphomas have also been described. Data supporting a clonal association with the underlying CLL are limited in these largely older case reports.
B-Cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia is a very rare disorder that is essentially defined by the morphologic features of the circulating monoclonal B cells, lacking both definitive immunophenotype and molecular/genetic features. Prolymphocytes, defined as cells with round nuclei, moderately condensed chromatin, and central prominent nucleoli, must exceed a threshold of 55% of lymphocytes, but in practice most cases exceed 90% [Campo 2008a]. Cases fulfilling these morphologic criteria are often linked to a clinical/hematologic profile that consists of age >60 years, marked leukocytosis, splenomegaly, and B symptoms [Campo 2008a, Del Giudice 2006]. The white blood cell (WBC) count generally exceeds $100 \times 10^3/\mu\text{L}$ ($100 \times 10^9/L$), consisting of a relatively homogeneous population of large cells with huge central nucleoli and moderate amounts of pale blue, agranular cytoplasm. Bone marrow examination generally reveals extensive effacement of normal hematopoietic cells and fat cells by the leukemic infiltrate, which often exhibits either a diffuse or a mixed nodular and diffuse pattern of infiltration on biopsy sections. These leukemic infiltrates are characterized by fairly widely spaced nuclei, prominent central nucleoli, and generally moderate mitotic activity; reticulin fibrosis may be present.

B-cell prolymphocytes exhibit a characteristic, though nonspecific, immunophenotypic profile, including strong monoclonal surface immunoglobulin expression, as well as expression of CD19, CD20, CD22, CD79a, CD79b, and FMC7, while either CD5 or CD23 expression is present in
only a minority of cases (10%-30%) [Campos 2008a]. Both CD38 and ZAP-70 are commonly expressed [Campos 2008a, Del Giudice 2006]. A diagnosis of MCL or CLL with prolymphocytoid transformation should be excluded in CD5+ cases. Indeed, one main focus of both immunophenotyping and cytogenetic assessment in cases of putative B-PLL is to exclude either leukemic MCL or CLL with prolymphocytoid transformation [Matutes 2004, Bachlemer 2004, Schlette 2003]. In addition to karyotype, cyclin D1 staining is helpful in identifying cases of MCL that mimic B-PLL.

There are no recurrent cytogenetic abnormalities in B-PLL, and complex cytogenetic abnormalities are common. Unlike CLL, neither genetics nor CD38/ZAP-70 expression is linked to survival [Campos 2008a, Del Giudice 2006]. Similarly, the presence or absence of somatic hypermutation is not predictive of outcome. As anticipated by the absence of either clear-cut molecular/
genetic or immunophenotypic features, the cell of origin of B-PLL is an unknown mature B cell [Camp 2008a].

Patients presenting with striking leukocytosis and marked organomegaly generally experience an aggressive disease course with median survival times ranging from 30-50 months. Unlike CLL, optimal staging and treatment strategies are not clear-cut, and treatment responses are generally poor, possibly the consequence of consistently detected p53 abnormalities [Camp 2008a].

**[22.4] Hairy Cell Leukemia**

**[22.4.1] Definition and General Features**

Hairy cell leukemia is an indolent mature B-cell neoplasm derived from cells with distinctive morphology (circumferential hairy projections) which exhibit a propensity for diffuse bone marrow and splenic red pulp infiltration [Foucar 2008]. Evidence supports an activated mature memory B cell of origin [Forcon 2004]. HCL is rare, accounting for <5% of chronic leukemias.

Hairy cell leukemia typically affects middle-aged to elderly patients (median age, 50 years), usually men (male-to-female ratio of 4:1), who present with splenomegaly and pancytopenia [Robak 2006]. HCL can occur as early as the third decade of life, but it is extremely unusual in patients <20 years of age [Foucar 2008]. Splenomegaly is a typical clinical feature in patients with HCL; however, because of earlier detection, the incidence of marked splenomegaly has declined. Hepatomegaly is variable but common, while most patients with HCL lack significant lymphadenopathy. Patients with HCL are often symptomatic, either from splenomegaly, producing left upper quadrant pain, or from the consequences of cytopenias (fever, fatigue, infection) [Hoffm 2006].

The clinical course of patients with HCL is generally indolent. Asymptomatic patients do not require therapy. In symptomatic patients, remarkable hematologic responses have been achieved using purine analog therapy (eg, 2-chlorodeoxyadenosine, cladribine, or 2-CDA) and other agents [Bel 2006, Robak 2006]. The overall 10-year survival rate exceeds 90%; median relapse-free survival times of 16 years have been reported [Chadha 2005, Else 2009].

**[22.4.2] Diagnosis of HCL**

Although HCL lacks a defining recurrent cytogenetic abnormality, this disease has numerous distinctive clinical and pathologic features. A list of prototypic clinical, hematologic, cyto/morphologic, and immunophenotypic features is provided in **[22.13]**. The successful diagnosis of HCL requires a high index of suspicion in conjunction with the integration of these diverse parameters.

### Prototypic Features of HCL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Older, male, splenomegaly, possible recurrent infections</td>
</tr>
<tr>
<td>Blood</td>
<td>Pancytopenia, profound monocytopenia, and neutropenia</td>
</tr>
<tr>
<td></td>
<td>Rare circulating hairy cells (inconspicuous)</td>
</tr>
<tr>
<td>Cytology</td>
<td>Round to oval nuclei with “spongy” chromatin; inconspicuous nucleoli</td>
</tr>
<tr>
<td></td>
<td>Abundant pale cytoplasm with circumferential projections</td>
</tr>
<tr>
<td>Cytochemistry</td>
<td>Strong tartrate-resistant acid phosphatase positivity (TRAP) (can also be assessed by immunohistochemistry)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Inaspirable in most cases due to diffuse reticulin fibrosis directly associated with hairy cell infiltrates</td>
</tr>
<tr>
<td></td>
<td>Subtle, patchy infiltrates on core biopsy (may be inconspicuous)</td>
</tr>
<tr>
<td>Flow cytometric immunophenotype</td>
<td>Monoclonal B cells with bright Slg, bright CD20, bright CD22, bright CD11c, bright CD103, and CD25</td>
</tr>
<tr>
<td></td>
<td>Subset CD10+</td>
</tr>
<tr>
<td>Immunohistochemical features</td>
<td>Positive for CD20, annexin-1, T-bet, CD123, DBA.44, TRAP; subset weakly cyclin D1+</td>
</tr>
</tbody>
</table>

HCL = hairy cell leukemia


**[22.4.3] Blood Findings**

Either single or multilineage cytopenias dominate blood findings in patients with HCL [Sharpe 2006]. Leukopenia, characterized by both neutropenia and marked monocytopenia, is the most common abnormality. Anemia is also a frequent finding in these patients. Erythrocytes are generally normocytic and normochromic, and the cause of anemia is multifactorial, including bone marrow production failure, splenic sequestration, and dilutional anemia secondary to increased plasma volume. Although characteristically inconspicuous, low numbers of hairy cells can often be identified on peripheral blood smears. A high index of suspicion is essential, and areas to include in scanning of the blood smear include the feather edge and thicker regions on the slides. Aside from reduced numbers, there are no qualitative abnormalities of neutrophils, monocytes, or platelets, a useful finding in distinguishing HCL from myelodysplasia.
Morphologic and Cytochemical Features on Smear Preparations

The nuclear and cytoplasmic features of classic hairy cells on smear preparations are well-delineated [22.13] [Foucar 2008, Sharpe 2006]. The nuclei of prototypic hairy cells range from round to oval, containing homogeneous, spongy, ground-glass chromatin with absent or inconspicuous nucleoli [22.45], [22.46]. The cytoplasm is abundant to voluminous, slate blue, and contains poorly delineated, patchy variations in the intensity of cytoplasmic staining sometimes described as flocculent. Occasional vacuoles, granules, and rod-shaped inclusions may be identified. These rod-shaped inclusions correspond to the ribosome lamellar complexes identified in ultrastructural studies [Cawley 2006]. The cytoplasmic membrane is characterized by numerous hairy projections, which are generally uniformly distributed around the circumference.

When aspiration is successful, abundant mast cells are often admixed with hairy cell infiltrates [22.13] [Macon 1993]. The disorder formerly designated as HCL-variant is categorized by the 2008 WHO criteria as a type of splenic diffuse red pulp B-cell lymphoma, and its relationship to HCL has been questioned (see [22.48] “Diagnostic Criteria and Differential Diagnosis,” p 499) [Piris 2008]. However, several other bona fide morphologically distinctive variants of HCL have been described. Despite the unusual morphologic characteristics, the diagnosis of HCL can be established in these cases by integrating cytochemical stain reactions, immunophenotypic features, clinical findings, and patterns of either bone marrow or splenic infiltration. These morphologic variants of HCL include convoluted, multilobulated, blastic, and spindled
cell variants; all are infrequently encountered in clinical practice [Bartl 1983, Catovsky 1984, Diez Martin 1987, Hanson 1989, Nazeer 1997]. These morphologically unique subtypes of HCL can usually be identified on both smears and sections. In both the convoluted and multilobulated subtypes, nuclear irregularity is frequent with lobulated, folded, cerebriform configurations i22.48. The blastic variant is characterized by an oval or indented nuclear contour, a finely dispersed nuclear chromatin pattern, and a central nucleolus i22.49. Elongated or spindled hairy cells may be evident on smears but tend to be more prominent on tissue biopsy sections (see next section). In all of these hairy cell variants, the cytoplasmic, cytochemical, and immunophenotypic features of prototypic HCL are retained.

The strong, fairly uniform reaction of hairy cells with tartrate-resistant acid phosphatase (TRAP) stains is useful in the diagnosis of HCL, even though the cytochemical stain has been replaced by the immunohistochemical method in many laboratories [Janckila 1995, Went 2005]. In most cases, a classic intense, diffuse cytoplasmic reaction product is identified in at least a subset of the leukemic cells i22.50. Cases of HCL that fail to exhibit any TRAP reactivity are very rare, ranging from 1%-5% in published series. Both normal myeloid and histiocytic cells and other neoplasms including leukemias, lymphomas, and mast cell disease can all exhibit some degree of TRAP positivity [Hoyer 1997, Janckila 1995]. However, if morphologic features, the intensity of the TRAP reaction product, and immunophenotypic findings are considered, cases of HCL can generally be distinguished from these other neoplastic and non-neoplastic TRAP+ cell types.

[22.4.5] Bone Marrow Biopsy Findings

Because of the very high frequency of unsuccessful aspiration, the bone marrow core biopsy is invaluable in establishing a diagnosis of HCL, despite the variation in both the pattern and extent of HCL infiltrates evident on these sections [Foucar 2008, Sharpe 2006]. The patterns of bone marrow involvement in HCL include subtle patchy infiltrates, diffuse interstitial infiltrates, and diffuse solid infiltrates that completely efface fat and hematopoietic cells. The patchy infiltrates are very poorly delineated from surrounding hematopoietic cells, often requiring both a high index of suspicion and very careful morphologic review for their identification i22.51. These subtle infiltrates can be highlighted by immunoperoxidase staining using pan B-cell antibodies and many other more HCL-specific/characteristic antibodies (see next section). The readily identifiable discrete lesions that are characteristic of bone marrow involvement by many other lymphoproliferative neoplasms are not a typical feature of bone marrow involvement by HCL i22.52. Sinusoidal
infiltration in HCL is fairly common, but this distinctive pattern of infiltration is admixed with the diffuse infiltrates and is often subtle.

Early reports on the morphology of bone marrow biopsy involvement by HCL emphasized the “fried egg” appearance of these infiltrates. This appearance resulted from the wide spacing of the nuclei secondary to the abundant cytoplasm of the hairy cells, distinct cytoplasmic membranes, and cleared-out cytoplasm [Bouroncle 1994, Hounieu 1992]. With the subsequent development of improved fixation techniques, this “fried egg” appearance is much less conspicuous. The nuclei, however, are still widely spaced, round to oval, and exhibit very little mitotic activity. The cytoplasm is moderate to abundant [i22.54].

A spectrum analogous to the variant morphologic forms of hairy cells described on aspirate smears can be identified on well-fixed and thinly sectioned bone marrow biopsy specimens [i22.55, i22.56, i22.57]. Good correlation exists between the identification of blastic and convoluted morphologic characteristics on both aspirate smears and biopsy sections. In addition, the spindled cell and osteosclerotic variants of HCL can be identified exclusively on biopsy sections [i22.56, i22.57].

Fibrosis in HCL

There is a consistent association between hairy cell infiltrates and reticulin fibrosis in virtually all organs studied, accounting for the high rate of aspiration failure in this disorder. Factors released by hairy cells such as fibronectin and/or transforming growth factor β1 are the likely cause of this fibrosis [i22.58] [Aziz 2003, Shehata 2004, Tiacci 2006]. The pattern of fibrosis varies from case to case, paralleling the extent of hairy cell infiltrates. For example, in cases of HCL with minimal patchy infiltrates, the reticulin fibrosis is localized to these minute foci of bone marrow involvement, while diffuse infiltrates are associated with extensive diffuse reticulin fibrosis. Rare cases of HCL are associated with both reticulin fibrosis and osteosclerosis; both lytic and blastic lesions have been noted radiographically [Filippi 2007, VanderMolen 1989].

Unusual Mast Cell and Vascular Lesions

Several additional findings noted on some bone marrow biopsy sections from patients with HCL include increased numbers of mast cells and vascular lesions [Macon 1993]. Increased numbers of mast cells are consistently identified on aspirate smears; these cells are also sometimes evident on biopsy sections. In rare cases, these mast cell aggregates are distinctive enough on biopsy sections to suggest concordant HCL and systemic mastocytosis [i22.59]. Even less commonly, increased numbers of dilated vascular spaces producing angiomatous lesions may also be evident on bone marrow biopsy sections from patients with HCL reminiscent of the vascular lakes described in the spleen [i22.60].
Convoluted hairy cell leukemia is illustrated on this bone marrow biopsy section. (H&E)

This bone marrow core biopsy section is from a 64-year-old female with profound pancytopenia. The specimen shows osteosclerosis along with a relatively subtle infiltrate of hairy cell leukemia, which was highlighted on immunohistochemical staining. (H&E)

Bone marrow biopsy sections illustrate a case of spindled hairy cell leukemia, which can closely mimic systemic mastocytosis. (H&E)

The spectrum of reticulin fibrosis on bone marrow biopsy sections from patients with hairy cell leukemia (HCL) is illustrated. When fibrosis is patchy, it tracks with patchy HCL infiltrates A. (reticulin)

A prominent perivascular infiltrate containing abundant mast cells is present on this bone marrow biopsy section from a patient with hairy cell leukemia. (H&E)

Bone marrow biopsy sections illustrate the unique focal angiomatous pattern present in this case of hairy cell leukemia. (H&E)
Another unique feature of HCL on bone marrow biopsy sections is the marked variability of cellularity of residual hematopoietic cells, even when hairy cell infiltration is minimal [22.61]. Up to 1/3 of cases of HCL exhibit marked bone marrow hypocellularity mimicking aplastic anemia [Foucar 2008, Sharpe 2006]. In these cases of hypocellular HCL, the granulocytic lineage is more potently suppressed than either the erythroid or megakaryocytic cell lines. Several publications suggest that inhibition of normal hematopoiesis in these patients is the consequence of either excess cytokine production (notably tumor necrosis factor α or transforming growth factor β1) by the hairy cells or inadequate colony-stimulating factor production by the bone marrow microenvironment [Casley 2006, Tiscit 2006]. Immunoperoxidase staining for CD20 is an essential screening stain used to identify the subtle B-cell infiltrate in these hypocellular specimens [22.62]. Once the occult B-cell infiltrate has been identified, further immunohistochemical stains are warranted (see below and 22.14).

### Immunohistochemical Studies in HCL

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reaction Pattern</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Surface membrane</td>
<td>Best marker for screening specimens for occult B-cell infiltrates</td>
</tr>
<tr>
<td>CD79a</td>
<td>Cytoplasmic</td>
<td>“Back-up” stain for CD20 in detecting occult B-cell infiltrates</td>
</tr>
<tr>
<td>Annexin 1</td>
<td>Cytoplasmic</td>
<td>Antibody identified by gene expression profiling</td>
</tr>
<tr>
<td>T-bet</td>
<td>Nuclear</td>
<td>T-cell associated transcription factor</td>
</tr>
<tr>
<td>CD123</td>
<td>Surface membrane</td>
<td>Expressed by variety of normal hematopoietic cells, acute leukemias, and HCL</td>
</tr>
<tr>
<td>DBA.44</td>
<td>Surface membrane (highlights hairy projections)</td>
<td>Positive in HCL, but also positive in other B-CLPN</td>
</tr>
<tr>
<td>TRAP</td>
<td>Cytoplasmic granules</td>
<td>Expressed in variety of neoplasms and benign histiocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most useful when combined with other antibodies</td>
</tr>
</tbody>
</table>

B-CLPN = B-chronic lymphoproliferative neoplasms; HCL = hairy cell leukemia; SMZL = splenic marginal zone lymphoma; TRAP = tartrate-resistant acid phosphatase cytochemical stain

[22.4.6] **Immunophenotypic Analysis**

A variety of B-cell antigens are expressed on the surface of hairy cells, including strong monotypic surface immunoglobulin, CD19, CD20, CD103, CD25, CD11c, and CD22, while CD79b, CD5, and CD23 are weakly expressed or absent [Foucar 2008, Matutes 2006]. By employing multicolor flow cytometric techniques, characteristic patterns of surface antigen expression typify HCL [Dong 2009, Matutes 2006]. Intense coexpression of CD22 and CD11c is a highly sensitive and specific immunophenotypic marker for hairy cells as is strong expression of the adhesion molecule CD103 and the interleukin-2 receptor (CD25) [Dong 2009, Matutes 2006]. However, since a significant proportion of cases of CLL, PLL, and even non-Hodgkin lymphomas also demonstrate weak to moderate coexpression of CD11c and CD22, evaluation of histograms for intensity of antigen expression is essential. Immunophenotypic variations by flow cytometric immunophenotyping of HCL include lack of CD103 (6% of cases), absence of CD25 (3% of cases), and expression of CD10 (10%-20% of cases) [Chen 2006, Dong 2009, Jasionowski 2003].

In addition to these flow cytometric techniques on cell suspensions, numerous antibodies with varying degrees of specificity are commercially available for paraffin-embedded immunohistochemical analyses [Del Giudice 2004, Falini 2004, Johrens 2007, Went 2005]. These immunohistochemical stains are highly useful for both the initial diagnosis on aspirable specimens as well as monitoring for response to therapy and minimal residual disease.

[22.4.7] **Molecular Genetic Features**

Molecular studies reveal immunoglobulin heavy-chain gene rearrangement in virtually all cases of HCL, and somatic hypermutation is characteristically indicative of a postgerminal center stage of maturation [Cawley 2006, Forconi 2004, Tucci 2006]. Cases of HCL with germline IGHV have been linked to refractoriness to therapy and a more aggressive disease course [Forconi 2009]. There are no recurrent cytogenetic abnormalities in HCL; numerical abnormalities of chromosomes 5 and 7 have been described, while translocations are uncommon [Cawley 2006, Foucar 2008].

[22.4.8] **Diagnostic Criteria and Differential Diagnosis**

Depending on the parameter being evaluated, the differential diagnosis of HCL is very broad. For example, some disorders, such as primary myelofibrosis, mimic the classic clinical features of HCL (eg, splenomegaly in an elderly man). Because of the broad morphologic and immunophenotypic spectrum of HCL, many other lymphoproliferative neoplasms may exhibit some features that mimic HCL. When classic hairy cells are present in abundance, they are usually easily recognized. However, because hairy cells are generally inconspicuous in blood and the bone marrow is often aspirable, the recognition of HCL can be challenging. This diagnostic challenge is further...
complicated by the variant forms of HCL that overlap with other B-CLPNs. Splenomegalic disorders such as splenic marginal zone lymphomas, B-PLL, splenic B-cell leukemias, unclassifiable (including HCL-variant), and splenomegalic MCL show substantial overlap of both morphologic and immunophenotypic features with HCL (see later sections for images). These disorders comprise the primary differential diagnostic considerations in HCL i22.15. However, rare cases of either plasma cell leukemia or myeloma can closely mimic HCL i22.64.

Possible Clonal Transformations

There is an increased incidence of second lymphoid neoplasms in patients with HCL. These include both B- and T-cell neoplasms, but B-cell disorders predominate. Although few molecular studies have been performed, an overlap of morphologic and immunophenotypic features suggest that at least some B-cell disorders identified in patients with
longstanding HCL may represent clonal transformations of the HCL [Nazeer 1997, Sun 2004]. Likewise, morphologic features suggesting 2 distinct lymphoid processes are occasionally identified on bone marrow biopsy sections of cases of HCL [22.66].

**Peripheralizing B-Cell Lymphomas**

**[22.5]** Splenic Marginal Zone Lymphoma (SMZL)

Even though SMZL is a recognized subtype of non-Hodgkin lymphoma, this disorder is included in this chapter because a leukemic blood picture is typical at presentation (see Chapter 24 for a more detailed discussion). This type of non-Hodgkin lymphoma affects elderly men who present with symptoms secondary to splenomegaly. Peripheral blood examination reveals mild anemia and/or thrombocytopenia, while the WBC count is moderately elevated, rarely exceeding $40 \times 10^3/\muL$ ($40 \times 10^9/L$)[Isaacson 2008, Matutes 2006, 2008]. Cytologic heterogeneity is evident on blood and bone marrow aspirate smears. Overall cell size and amount of cytoplasm are variable. Some cells are typically plasmacytoid with bipolar cytoplasmic projections, while others exhibit HCL-like circumferential projections [22.67, 22.68].

The immunophenotypic profile of SMZL is provided in [22.8] and [22.15][Campo 2008a, Foucar 2008, Isaacson 2008, Matutes 2004, 2006, 2008, Piris 2008, Ruchlemer 2004]. On bone marrow biopsy sections, the infiltrate is characteristically nodular with readily apparent infiltrates at low
magnification i22.69, i22.70. In addition, intrasinusoidal invasion is a common and distinctive feature of SMZL that can be highlighted by immunohistochemical staining i22.71 [Isaacson 2008, Matutes 2006, 2008]. The disease course of SMZL is indolent, and some patients may not initially require therapy.

i22.5.2 Hairy Cell Leukemia-Variant (HCL-v)
Although the acronym HCL-v implies a relationship to classical HCL, this rare disorder is currently considered to be a subtype of splenic B-cell leukemia, unclassifiable [Piris 2008]. In this rare, poorly defined disorder, the cytologic features of the circulating cells in the blood typically show hybrid features between classical HCL and B-PLL i22.15 i22.72 [Matutes 2003, Piris 2008]. In addition to splenomegaly, leukocytosis is common, and, unlike classical HCL, monocytes are present in normal numbers. Cytologic features on blood smears may be quite variable with a range in cell types. Nuclei are typically round, but may be convoluted, and nucleoli are generally prominent. Cytoplasmic projections are usually present, but variable i22.73. Bone marrow infiltrates may be subtle with a predilection for sinusoidal infiltration i22.74 [Dong 2009, Matutes 2003, Ya-In 2005]. The immunophenotype is also variable, but the classical HCL “profile” is lacking in that CD25, annexin-1, CD123, and TRAP are characteristically negative [Cesana 2005, Dong 2009]. VH4-34 gene usage is common in cases classified as HCL-v and is linked to unmutated IGHV and adverse outcomes [Arons 2009].

i22.5.3 Other NHLs with Leukemic Picture
In addition to SMZL, peripheralizing lymphoma cells can be identified in a substantial proportion of patients with other
types of NHL. However, in contrast to SMZL, the peripheral blood involvement in other types of NHL does not usually produce a de novo leukemic blood picture, except for the splenomegalic subtype of MCL. Likewise, peripheralization of MCL can also occur later in the disease course in a fair proportion of cases. In both de novo leukemic MCL and cases in which peripheralization is a late feature, a morphologic spectrum has been described.

Some cases exhibit a mature nuclear chromatin configuration with moderate nuclear irregularity, while other cases have a distinctly blastic appearance with variably prominent nucleoli; overlap with prolymphocytic leukemia and even acute leukemia is common.

For most other types of NHL, the identification of substantial numbers of circulating lymphoma cells occurs in association with longstanding NHL and extensive bone marrow replacement. In these patients, the blood-bone marrow barrier is thought to be disrupted by the neoplastic infiltrate, resulting in the release of these cells into the blood. Circulating lymphoma cells are generally morphologically similar to those seen on bone marrow aspirate smears. Although small cell lymphomas such as follicular and mantle cell lymphomas account for the majority of peripheralized lymphomas, both lymphoblastic and large cell lymphoma cells may also circulate (see also Chapter 24). In ~10% of patients with NHL, a frank leukemic peripheral blood picture develops in which a striking leukocytosis is evident. On immunophenotyping studies, the majority of peripheralized lymphomas are of B-cell type, and follicular lymphomas demonstrating coexpression of CD10 predominate. Peripheralized MCLs exhibit...
immunophenotypic and genotypic features analogous to the more common nodal forms of this disease [22.8, 22.6] [Matutes 2004]. See [22.8, 22.9, 22.10, 22.11] for comparisons of B-CLPN in blood, bone marrow, and on immunophenotyping.

![Peripheralizing mantle cell lymphoma characterized by bright CD20 and CD5 coexpression in addition to CD23 expression is evident on this flow cytometric histogram composite from peripheral blood. Neoplastic cells are circled.](image)

### [22.6] T-Chronic Lymphoproliferative Neoplasms

Analogous to B-CLPN, there are several types of T-chronic lymphoproliferative neoplasms (T-CLPN) with leukemic manifestations, including T-cell large granular lymphocytic leukemia (T-LGL), T-cell prolymphocytic leukemia (T-PLL), Sézary syndrome (SS), and adult T-cell leukemia lymphoma (ATLL). These mature T-CLPN often manifest as systemic disorders with splenomegaly with or without hepatomegaly, skin lesions, and variable lymphadenopathy in conjunction with hematologic abnormalities [Foucar 2007]. The focus of this chapter will be the blood and bone marrow manifestations of these disorders. Note that both non-neoplastic and neoplastic disorders of true NK cells are the focus of Chapter 26. Mature T-cell leukemias can be broadly defined as clonal proliferations of morphologically and immunophenotypically mature T cells of either helper (CD4+) or cytotoxic/suppressor (CD8+) type.

Clinical, hematologic, morphologic, immunophenotypic, and selected genetic features are essential in the subclassification of T-CLPN [22.16]. Based on the integration

### [22.16] Key Features of Mature T-Cell Leukemias

<table>
<thead>
<tr>
<th>Feature</th>
<th>T-LGL</th>
<th>T-PLL</th>
<th>SS</th>
<th>ATLL (acute)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td>Wide age range, no sex predilection</td>
<td>Elderly, male predominance</td>
<td>Middle age to elderly, male predominance</td>
<td>Endemic HTLV-1 associated leukemia</td>
</tr>
<tr>
<td></td>
<td>Symptoms linked to cytopenias, splenomegaly common</td>
<td>Hepatosplenomegaly with generalized lymphadenopathy</td>
<td>Cutaneous disease, especially generalized erythroderma</td>
<td>Occurs in minority of carriers after long latency period</td>
</tr>
<tr>
<td></td>
<td>Association with rheumatoid arthritis, recurrent infections and red cell aplasia</td>
<td>Skin lesions and pleural effusions in subset</td>
<td>Generalized lymphadenopathy common</td>
<td>Aggressive multisystem disease</td>
</tr>
<tr>
<td><strong>CBC findings</strong></td>
<td>Absolute lymphocyte count 2-20 × 10^3/μL (2-20 × 10^9/L; sustained &gt;6 months)</td>
<td>Marked lymphocytosis (&gt;100 × 10^3/μL &gt;100 × 10^9/L)</td>
<td>Variable lymphocytosis</td>
<td>Variable lymphocytosis, often striking</td>
</tr>
<tr>
<td></td>
<td>Variably severe anemia and/or neutropenia</td>
<td>Rapidly rising WBC</td>
<td>Variable cytopenias</td>
<td>Variable cytopenias</td>
</tr>
<tr>
<td><strong>Morphology of lymphocytes</strong></td>
<td>Round nuclei, condensed chromatin, moderate amounts of cytoplasm with distinct granules</td>
<td>Spectrum, variably prominent nucleoli, variable nuclear irregularity</td>
<td>Anemia and thrombocytopenia common</td>
<td>Eosinophilia common</td>
</tr>
<tr>
<td></td>
<td>Granules contain granzyme and perforin</td>
<td>In cell size Subtle nuclear convolutions</td>
<td>Spectrum in size and nuclear configurations Pronounced nuclear lobulation (cloverleaf)</td>
<td></td>
</tr>
<tr>
<td><strong>Key immunophenotypic features</strong></td>
<td>Pan-T+*, TCR α/β, CD8, CD57</td>
<td>Pan-T+*, TCR α/β Usually CD4, but CD4/CD8 coexpression in 1/4</td>
<td>Pan-T+*, TCR α/β CD4, variable CD25, reduced or absent CD7</td>
<td>Pan-T+*, TCR α/β CD4+, CD25+ Reduced or absent CD7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCL-1 (nuclear &amp; cytoplasmic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genetic features</strong></td>
<td>TCR gene rearrangement + (may be useful in distinguishing from reactive LGLs)</td>
<td>inv(14)(q11q23) results in TCL-1 translocation to promoter region of TCR genes (other translocations described)</td>
<td>No recurrent cytogenetic findings</td>
<td>No recurrent cytogenetic findings</td>
</tr>
</tbody>
</table>

*Some pan T-cell antigens may exhibit aberrant reduced expression

†Other less common associations include myelodysplasia and aplastic anemia

T-LGL = T-cell large granular lymphocytic leukemia; T-PLL = T-cell prolymphocytic leukemia; SS = Sézary syndrome; ATLL = adult T-cell leukemia/lymphoma; CBC = complete blood count; TCL = T-cell leukemia; HTLV = human T-cell leukemia virus; TCR = T-cell receptor; WBC = white blood cell

of multiple parameters, most cases of neoplastic T-cell disorders in blood can be subclassified into the 4 disorders encompassed in this chapter. However, the diagnostician must be aware that other peripheral T-cell lymphomas may occasionally manifest in blood (see "Differential Diagnosis," p 507 and Chapter 24).

**Morphologic Features**

In most patients with T-LGL, the blood contains increased numbers of morphologically mature, generally normal-appearing LGLs [22.78, 22.79] [Chan 2008, O’Malley 2007]. Occasionally the clonal cells of T-LGL exhibit more pleomorphism and/or immaturity [Alekshun 2007]. Other common blood features include neutropenia and anemia. Although bone marrow examination is often not necessary, especially in patients with stable indolent disease, bone marrow findings in T-LGL are well delineated [Chan 2008, Evans 2000, O’Malley 2007, Osuji 2007]. It is important for the diagnostician to be aware that T-LGL infiltrates in the bone marrow are subtle, poorly delineated, and patchy [22.80, 22.81, 22.82]. Consequently, immunohistochemical assessment is critical [Osuji 2007]. In patients with a more aggressive T-LGL, the bone marrow may be effaced by a diffuse infiltrate of leukemic cells [Alekshun 2007]. Overall bone marrow cellularity is often increased, especially in patients with myeloid expansion as a response to neutropenia [Burks 2006, Osuji 2007].

Although lymphoid aggregates may be readily apparent on H&E-stained sections, these lymphoid cells are characteristically non-neoplastic based on morphology and immunohistochemistry [Osuji 2007]. Other nonspecific features include reticulin fibrosis, eosinophilia, and megakaryocytic hyperplasia. Assessment for T-LGL-associated red cell aplasia and myelodysplasia is essential [22.83] [Epling-Burnett e 2007, Huh 2009]. Consequently, each hematopoietic lineage must...
22.78 This peripheral blood smear from a 73-year-old female with T-cell large granular lymphocytic leukemia shows a marked increase in circulating mature-appearing large granular lymphocytes. (Wright) (courtesy Q-Y Zhang)

22.79 This peripheral blood smear from a 73-year-old patient with T-cell large granular lymphocytic leukemia shows prominent cytoplasmic granules in the majority of circulating neoplastic cells. Note binucleation and occasional shaggy cytoplasmic contours. (Wright)

22.80 This bone marrow aspirate smear from this patient with T-cell large granular lymphocytic leukemia shows an increase in mature-appearing lymphoid cells, which exhibit nondescript morphologic features. (Wright)

22.81 Low magnification of a bone marrow core biopsy section from a patient with T-cell large granular lymphocytic leukemia shows subtle areas of increased cellularity, but no discrete lymphoid infiltrates are appreciated. (H&E)

22.82 On high magnification, the subtle lymphoid infiltration in this case of T-cell large granular lymphocytic leukemia is more apparent (see 22.81). The areas of increased cellularity noted at low magnification consist largely of mature, lymphoid-appearing cells with widely spaced nuclei compatible with moderate amounts of cytoplasm. (H&E)

22.83 Immunoperoxidase staining for hemoglobin A on this bone marrow core biopsy section shows only widely scattered erythroblasts in this patient with T-cell large granular lymphocytic leukemia and associated red cell aplasia. (immunoperoxidase for hemoglobin A)
be systematically assessed for quantitative and qualitative abnormalities.

Immunoperoxidase staining of the bone marrow biopsy sections for T-cell antigens (CD3, CD8, TIA-1, CD57) can highlight subtle infiltrates. Immunohistochemical staining is particularly useful in highlighting sinusoidal infiltrates of T-LGL [Morice 2002, O’Malley 2007, Osuji 2007]. TIA-1 stains the intracytoplasmic granules of LGLs and is thus much less conspicuous on immunohistochemical staining.

**Immunophenotypic and Molecular Analyses**

The classic flow cytometric T-LGL profile consists of expression of surface CD3, CD2, CD5, CD8, CD16, and CD57 [22.7], but anomalies in the intensity of antigen expression are common [Chan WC 2008, Lundell 2005]. Clonal T-cell receptor gene rearrangement is characteristically identified. Although various numerical and structural cytogenetic abnormalities have been described, there are no known recurrent cytogenetic abnormalities in T-LGL [Chan WC 2008]. Rare reports describe CD4+, CD56+, CD57+ clonal proliferations of large granular lymphocytes [Oltbrunn 2008].

**Differential Diagnosis**

The distinction between chronic LGL disorders and other mature T-cell leukemias is generally not a problem, since both granulated lymphocyte morphology and the cytotoxic suppressor immunophenotype are unique to T-LGL. More problematic is the distinction between reactive and neoplastic T-LGL proliferations; molecular assessment for T-cell receptor gene rearrangement is particularly useful in this...
setting. Similarly, immunophenotypic assessment is essential in the distinction between T-LGL and chronic lymphoproliferative disorders of NK cells, which can be morphologically identical (see Chapter 26 for images) [Chan 2008, Villanmer 2008].

22.6.2 T-Cell Prolymphocytic Leukemia

General Features

Although controversial, the current recommendation is to combine disorders previously termed T-PLL and T-CLL into a single disease category. Because of similarities in both clinical course and cytogenetic abnormalities, T-CLL may be best viewed as a small cell variant of T-PLL [Catovsky 2008]. Like patients with B-PLL, most patients with T-PLL are elderly men who present with marked leukocytosis and pronounced splenomegaly [22.16]. However, patients with T-PLL more commonly exhibit lymphadenopathy, cutaneous involvement, and pleural effusions than those with B-PLL [Catovsky 2008, Foucar 2007].

Blood Findings

Although the morphologic features tend to be homogeneous within individual cases, a spectrum of morphologic features has been noted in T-PLL. In some cases the cells are similar to those seen in B-PLL, exhibiting round nuclear contours with huge central nucleoli. In other cases the leukemic cells exhibit greater nuclear irregularities, often appearing knobby with cytoplasmic protrusions and blebs [22.86, 22.87, 22.88]. In occasional T-PLL cases, the nuclear irregularity approaches that seen in SS or ATLL. The nuclear chromatin is generally condensed, and nucleoli are variably prominent but sometimes inconspicuous. The relatively scant cytoplasm is basophilic and agranular and may exhibit prominent blebs. One distinctive hematologic feature in T-PLL is a rapidly rising WBC [Herling 2004]. Other common hematologic features include anemia and thrombocytopenia.

Bone marrow infiltrates of T-PLL are characteristically diffuse with extensive replacement of hematopoietic cells and fat cells [22.89] [Catovsky 2008, Viswanatha 2003]. Occasional cases exhibit a mixed nodular and diffuse pattern of infiltration, and fibrosis has sometimes been associated with these leukemic infiltrates [Nito 1989]. Despite this mild fibrosis, numerous prolymphocytes are generally evident on bone marrow aspirate smears, and these atypical cells exhibit the same cytologic features as the circulating neoplastic cells.

Immunophenotypic and Genetic Features

T-prolymphocytic leukemia is derived from immunophenotypically mature T cells that express the pan T-cell antigens, CD2, CD3, CD5, and CD7 [22.16]. The majority of cases exhibit a helper cell phenotype, although one fourth of cases of T-PLL demonstrate coexpression of both CD4 and CD8, while others exclusively express CD8 [Catovsky 2008, Foucar 2007]. Despite morphologic heterogeneity, a consistent cytogenetic abnormality has been identified in 80% of cases of T-PLL. This abnormality is inversion (14)(q11q32) [22.8]; this translocation juxtaposes TCL1 oncogene into the promoter region of the T-cell receptor α/δ gene. Constitutive dysregulation of TCL1 is leukemogenic [Catovsky 2008, Pékarský 2004]. Consequently, immunohistochemical staining for TCL1 shows both nuclear and cytoplasmic positivity in T-PLL and is absent in other T-CLPN [22.90] [Herling 2004, 2008]. Other cytogenetic abnormalities in T-PLL have

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[i22.86] This peripheral blood smear from a 36-year-old male with a white blood cell count higher than 350 × 10⁹/μL (350 × 10⁹/L) shows a striking mature lymphocytosis that on immunophenotyping was immunologically mature, but coexpressed CD4 and CD8 as often seen in T-cell prolymphocytic leukemia. (Wright)

[i22.87] This peripheral blood smear is from an 89-year-old female with T-cell large granular lymphocytic leukemia who presented with a skin rash. Complete blood count showed a white blood cell count of 330 × 10⁹/μL (330 × 10⁹/L). These cells were confirmed to be mature T cells by immunophenotyping. Note generally prominent central nucleoli resembling B-cell prolymphocytic leukemia. (Wright)
been described, some of which result in homologous oncogene dysregulation, while others are linked to deletion of ATM tumor suppressor gene [Catovsky 2008]. The disease course of T-PLL is typically aggressive but improved response rates have been achieved with anti-CD52 therapy [Catovsky 2008].

Differential Diagnosis

The primary differential diagnostic challenge is the distinction of T-PLL from other mature T-CLPNs (22.16). Features useful in identifying cases of T-PLL include the marked and rapidly rising leukocytosis, massive splenomegaly, strong expression of CD7, TCL-1 expression, and the unique genetic features, all features absent in other CD4 positive T-CLPN.

### Sézary Syndrome

**General Features**

Sézary syndrome and mycosis fungoides (MF) are closely related, but pathophysiologically distinct, disorders that compose the bulk of cutaneous T-cell lymphomas [Ralfkiaer 2008a, 2008b; van Doom 2009]. Both disorders are derived from mature T lymphocytes that express the helper antigen, CD4. These cells are morphologically unique in that they exhibit a high nuclear-cytoplasmic ratio with marked nuclear convolutions resulting in a cerebriform appearance. The distinction between SS and MF is based largely on the pattern of cutaneous disease. Patients categorized as having SS have generalized exfoliative erythroderma with characteristic blood involvement, while patients with MF tend to have
prolonged cutaneous disease with a very wide spectrum of cutaneous lesions ranging from plaques to tumor nodules. Consequently, this chapter focuses largely on the blood and bone marrow findings in SS, the more aggressive variant of cutaneous T-cell lymphoma [Ralfkiaer 2008b].

Blood Findings

Although nonspecific findings such as eosinophilia and anemia of chronic disease may be evident, the major focus of blood examination in patients with SS is to identify circulating malignant T cells. By convention, these circulating cells are often referred to as Sézary cells, and these cells may be identified by either morphologic appearance or immunophenotypic properties, although immunophenotype is much more reliable [Morice 2006, Vonderheid 2006]. The incidence of blood involvement in patients with generalized erythroderma (SS) approaches 100%.

Despite the prominent variation in the size of these circulating atypical lymphoid cells, the nuclei of these cells are cerebriform with variably condensed nuclear chromatin, while nucleoli are absent or inconspicuous i22.91, i22.92, i22.93, i22.94. A predominance of large Sézary cells is linked to adverse outcome. The nuclear-cytoplasmic ratio is generally high, and the cytoplasm is typically basophilic and agranular. Occasional cytoplasmic vacuoles may be identified.

Because it may be difficult to morphologically identify circulating Sézary cells with certainty, especially when present in low numbers, many routine and specialized techniques have been applied to facilitate this process (see later section for specialized methods). Cytochemical staining may highlight globules of cytoplasmic periodic acid-Schiff-positive

**Blood smears illustrate circulating Sézary syndrome cells from 3 patients. Note condensed chromatin, subtle nuclear convolutions, and size range. (Wright)**

**This intermediate magnification of a peripheral blood smear from a patient with Sézary syndrome highlights the distinctly dark nuclear chromatin configuration that characterizes this disorder. Note broad size variation of circulating Sézary cells including small forms of approximately the size of a normal lymphocyte (lower left). (Wright) (courtesy B Nelson, MD)**

**Because of the subtle nuclear irregularities, Sézary cells can be confused with monocytes. The differences between a Sézary cell (center) and a monocyte (lower right) is highlighted in this peripheral blood smear from a patient with Sézary syndrome. (Wright)**

**The subtle cerebriform nuclear configuration of circulating Sézary cells is evident in this composite from 2 patients with Sézary syndrome. (Wright) (a courtesy B Nelson, MD).**
material, while the absence of nonspecific esterase activity can be used to distinguish Sézary cells from monocytes. Although electron microscopy is not utilized in routine practice, the cerebriform nuclear configuration of circulating Sézary cells is best appreciated by this method

**Bone Marrow Features**

Despite the high frequency of peripheral blood involvement, overt bone marrow infiltration by SS is much less frequent [Ralfkiaer 2008b]. Although some investigators report a 22% incidence of bone marrow infiltration by SS, these infiltrates are often very subtle and easily missed, identifiable only by a careful examination under oil immersion or immunohistochemical assessment [22.96, 22.97]. In cases with more extensive bone marrow involvement, SS cells can be identified on aspirate smears [22.98]. However, bone marrow biopsy sections are superior both for the identification of cells with markedly cerebriform nuclei and for assessing the pattern of infiltration (either small inconspicuous collections or interstitial infiltrates of isolated cerebriform cells) [22.96]. Immunohistochemical stains for CD3, CD4, CD7, and CD8 may be very helpful in highlighting CD3+, CD4+, CD7−, and CD8− interstitial infiltrates [22.97].

**Flow Cytometric Immunophenotypic Features**

Circulating Sézary cells exhibit the same mature helper T-cell phenotype demonstrated in cutaneous infiltrates [22.16]. In addition to CD4, these cells also express pan T-cell antigens such as CD2, CD3, and CD5, while CD7 is generally absent or weak [22.9] [Ralfkiaer 2008b]. Although multicolor flow cytometric immunophenotyping is being increasingly utilized to identify low numbers of circulating lymphoma cells in SS patients, the diagnostician must be aware that normal blood control samples and specimens from patients...

**Cytogenetic and Molecular Studies**

In stimulated peripheral blood specimens, clonal cytogenetic abnormalities are common, but there are no recurrent cytogenetic features in SS [Ralfkiaer 2008b]. Molecular studies, whether performed on involved skin, lymph node, or peripheral blood, document consistent T-cell receptor gene rearrangements in patients with SS.

**Differential Diagnosis**

The differential diagnosis of SS logically includes the other T-CLPNs. Subclassification among these uncommon leukemias depends on the integration of clinical, morphologic, and immunophenotypic data [22.16]. Distinction between non-neoplastic circulating T cells and minimal involvement by SS is problematic [Olsen 2007]. Parameters utilized to define blood involvement include flow cytometric immunophenotyping, molecular genetic analyses, and morphologic assessment [Olsen 2007]. Of these methodologies, morphologic assessment is the most subjective and shows the greatest interobserver variation [Olsen 2007]. The detection of a significant population of CD4+, CD7− T cells (exceeding laboratory normal reference ranges) is the main modality currently used in clinical practice to delineate the number of circulating SS [Morice 2006, Olsen 2007, Vonderheid 2006]. Loss of CD26 expression is also useful, as is a helper to suppressor ratio >10 [Morice 2006]. T-cell clonality is of most utility when combined with clonality assessment of other sites of disease (usually skin), since T-cell clones can be present in blood from patients with non-neoplastic disorders [Olsen 2007, Vonderheid 2006]. Rare reports of pseudoleukemic picture with features of SS following drug therapy have been published in patients without cutaneous T-cell lymphoma [Jabbar 1998].

**Adult T-Cell Leukemia/Lymphoma**

**General Features**

Adult T-cell leukemia/lymphoma is a distinctive mature T-cell neoplasm originally described in Japanese patients in 1977 [Ferreira 1997, Franchini 1995, Uchiyama 1977, 1988]. Subsequent studies have documented its unique worldwide geographic distribution, with the vast majority of cases occurring in Japan, Africa, the southeast United States, and the Caribbean region [Ohshima 2008, Pise-Masison 2009]. However, nonendemic cases have been described, and it is possible that ATLL has a more widespread distribution than generally recognized.

The etiologic agent causing endemic ATLL is the human T-cell leukemia virus type 1 (HTLV-1), which can be identified by serologic, immunologic, or molecular studies in virtually all endemic cases [Nicot 2005, Ohshima 2008]. However, only 2.5% of chronic HTLV-1 carriers develop overt malignancies, so infection alone is insufficient to induce neoplasia [Nicot 2005, Ohshima 2008, Pise-Masison 2009].

Although originally recognized as a leukemic disorder, the range of HTLV-1-related disease is broad and even includes a neurodegenerative disorder. For that subgroup of HTLV-1-infected patients who develop leukemia/lymphoma-type manifestations, a spectrum of clinical and pathologic findings has also been identified, ranging from asymptomatic patients with sustained mild lymphocytosis to those with florid leukemic or lymphomatous manifestations [Ohshima 2008, Yokote 2005]. The progression from an asymptomatic carrier state to a leukemic phase generally takes decades. Recent evidence suggests that gene expression profiling may be useful in distinguishing biologic subtypes of HTLV-1-related disorders [Pise-Masison 2009].

The most aggressive type of HTLV-1-associated leukemia/lymphoma has been designated as acute ATL, and only this leukemic variant will be presented in this chapter. Patients suffering from acute ATL present with a leukemic
blood picture, clinical manifestations of leukemia, often in conjunction with prominent extramedullary disease. These patients often have lymphadenopathy, hepatosplenomegaly, skin lesions, and lytic bone lesions; ~1/3 also have hypercalcemia [Ohshima 2008]. The clinical course is very aggressive, with short median survival times.

The focus of this chapter discussion is on the peripheral blood findings, bone marrow morphologic features, and immunophenotypic features of this acute type of ATL. Both endemic and nonendemic cases of acute ATL have been described in the United States. Because many of the nonendemic cases fail to demonstrate evidence of HTLV-1 infection, it is possible that another agent is responsible for this disease [Head 1987]. However, both the morphology and immunophenotype of these nonendemic cases are identical to those described in endemic acute ATL.

Peripheral Blood Findings

The most remarkable peripheral blood abnormality in acute ATL is the striking leukocytosis, with a predominance of morphologically abnormal lymphoid cells. These lymphoid cells generally show pronounced nuclear irregularity with coarse lobulations and cloverleaf forms, though cases resembling either CLL or PLL have been described [Foucar 2007]. Heterogeneity of both overall cell size and nuclear configurations is common. The chromatin of these cells tends to be coarsely clumped, and nucleoli are either absent or inconspicuous. The amount of cytoplasm varies from scant to moderate and is generally slightly basophilic and agranular. Both an associated eosinophilia and neutrophilia have been occasionally described in these patients, possibly the consequence of cytokines released from the leukemic cells. Hemoglobin and platelet counts vary, depending on the degree of bone marrow replacement by the leukemia.

Bone Marrow Findings

Patterns of bone marrow infiltration by acute ATL vary from patchy to focal paratrabeicular to extensive, diffuse replacement of the entire hematopoietic space. Occasionally, the bone marrow involvement is subtle, despite prominent blood involvement. On aspirate smears and tissue sections, these cells exhibit the same striking nuclear irregularities identified in the peripheral blood. Mitotic activity is generally not prominent. Eosinophilia may be associated with acute ATL infiltrates in bone marrow and other tissues [Ogata 1998]. In addition, striking bony abnormalities, consisting predominantly of increased numbers of osteoclasts and osteoblasts, are very frequently identified on bone marrow biopsy sections from these patients [Foucar 2007]. Increased bone resorption is
the cause of the hypercalcemia that is commonly identified in these patients. Constitutive production of parathyroid hormone-related protein by the leukemia cells is the primary cause of this pathologic finding [Nadella 2007].

**Immunophenotypic Findings**

In both endemic and nonendemic cases, ATLL cells demonstrate a consistent immunophenotypic profile [Ohshima 2008]. These cells represent mature T cells, lacking both terminal deoxynucleotidyl transferase and CD1 expression. Several pan T-cell antigens are expressed on ATLL cells, including CD3, CD2, and CD5, while CD7 expression is absent or weak [Foucar 2007, Ohshima 2008]. Reduced expression of CD3 was noted in about half of the cases in one study [Yokote 2005]. ATLL cells also commonly express activation antigens, most notably CD25. Most cases of ATLL demonstrate a helper cell (CD4+) phenotype; although small numbers of cases demonstrate coexpression of both CD4 and CD8, rare cases lack either CD4 or CD8, and other rare cases demonstrate only CD8 expression [Foucar 2007].

**Cytogenetic and Molecular Studies**

Although almost all case of ATLL exhibit cytogenetic abnormalities, none is specific [Ohshima 2008]. Molecular studies of ATLL cells consistently demonstrate clonal T-cell receptor gene rearrangement. In addition, HTLV-1 is clonally integrated in the neoplastic cells [Kamihira 2005, Ohshima 2008].

**Differential Diagnosis**

Despite the disorder’s rarity in the United States, the striking morphologic abnormalities of circulating acute ATL cells are so unique that cases can generally be suspected. Although both mature and blastic T-cell leukemias can exhibit circulating cells with nuclear irregularities, these disorders can generally be distinguished from acute ATL by integrating morphologic, clinical, viral, and immunophenotypic findings [22.16].

[22.7] **Components of the Diagnostic Interpretation in CLPN**

The goal in the assessment of a patient for a possible B- or T-CLPN is to first exclude any reactive process, followed by an effort to delineate the hematologic, morphologic, and immunophenotypic profile of the neoplastic clone [22.18]. This complex, multifaceted endeavor facilitates the identification of cases that fulfill diagnostic criteria for well-defined clinicopathologic entities. For cases that exhibit hybrid features, the pathologist should acknowledge diagnostic uncertainty and recommend additional tests that may allow definitive subclassification. For purposes of treatment, clinicians may require a “best fit” label, with the caveat that the case exhibits some aberrant properties. It is also useful to discuss those features known to have prognostic significance.

For both B- and T-cell neoplasms, peripheralized lymphomas are a key differential diagnostic consideration. Although lymphomas with typical “leukemic” features at presentation have been included in this chapter, other B- and T-cell lymphomas may manifest rarely with a leukemic blood picture. B-cell lymphomas to be considered include mantle cell, follicular, lymphoplasmacytic, and diffuse large B-cell subtypes. T-cell lymphomas that can occasionally manifest a leukemic blood picture include hepatosplenic T-cell, anaplastic large cell lymphoma, angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified (NOS) (see Chapter 24).

[22.8] **Clues and Caveats**

1. Current classification proposals for B- and T-cell CLPNs are based on the identification of distinct clinicopathologic entities by integrating clinical, morphologic, immunophenotypic, and often genotypic properties.

2. A leukemic blood and bone marrow picture is by definition a de novo feature of chronic leukemias, while a similar picture is noted occasionally in primarily lymphomatous disorders.

3. A spectrum of morphologic and immunophenotypic findings characterizes all CLPNs, and overlap among these disorders is common. As a consequence, precise subclassification may sometimes be problematic and arbitrary.
4. A “classic” immunophenotypic profile has been delineated for all B- and T-cell CLPNs, which takes into account pattern and intensity of antigen coexpression as well as antigens that are characteristically absent.

5. Immunophenotype and genotype provide both diagnostic and prognostic information in CLL.

6. Monoclonal B lymphocytosis is a precursor lesion to CLL.

7. The distinction between monoclonal B lymphocytosis and low-stage CLL is problematic; current recommendations for CLL require a sustained absolute monoclonal B-cell count of ≥5 × 10^9/μL (≥5 × 10^6/L) for 3 months.

8. The diagnosis of CLL based exclusively on flow cytometric immunophenotyping can be problematic when the absolute lymphocyte count by CBC is not known.

9. Most cases of CLL are composed of small monotonous lymphocytes with round nuclei and scant cytoplasm; “atypical” CLL is characterized by greater cytologic heterogeneity, including nuclear clefting and increased prolymphocytes.

10. In CLL, atypical morphologic features, aberrant immunophenotype, and disease progression/ transformation are often linked to specific genetic abnormalities.

11. In addition to disease stage, many other independent prognostic factors have been described in CLL, including CD38 expression, ZAP-70 expression, cytogenetic features, and IGHV mutation status.

12. Potential new prognostic factors such as microRNA profiles may become clinically useful.

13. Numerous T-cell and NK cell abnormalities have been noted in patients with B-CLL; even rare clonal T-cell defects have been described in these patients.

14. The distinction among CLL, prolymphocytoid transformation of CLL, and B-PLL is based primarily on the percentage of prolymphocytes, and the monotonity/heterogeneity of the neoplastic cells. Despite some immunophenotypic and genetic differences, there may be overlap.

15. Although prolymphocytes typically exhibit prominent nucleoli, nuclear chromatin is more condensed than that of blasts.

16. Many types of transformation have been described in CLL, but prolymphocytoid transformation and Richter syndrome (overt large cell lymphoma) are the most common.

17. The majority of large cell lymphomas that develop in patients with underlying CLL represent clonal transformations; a minority represent secondary, often EBV-associated neoplasms.

18. Rare cases of CLL with scattered Reed-Sternberg-like cells must be distinguished from overt Hodgkin-like transformation of CLL.

19. The nuclear chromatin of CLL cells is densely and coarsely clumped; chromatin is less condensed in prolymphocytes, while the nuclear chromatin of hairy cells is dispersed with a spongy, ground-glass appearance.

20. Discrete, focal, nodular bone marrow infiltrates are common in patients with CLL, B-PLL, and many lymphomas, but distinctly uncommon in HCL.

21. Strong, diffuse tartrate-resistant acid-phosphatase positivity is characteristic of HCL, while less intense reactivity may be present in many other disorders.

22. Other relatively HCL-specific markers include annexin-1, CD123, and t-bet.

23. Patchy infiltrates of hairy cells can be very subtle and are not sharply delineated from adjacent hematopoietic cells. Immunoperoxidase studies on bone marrow biopsy sections using pan B-cell antibodies can highlight these cells.

24. Hypocellular HCL is common and is probably the result of HCL-associated cytokine suppression of hematopoiesis.

25. Convoluted, multilobulated, plastic, and spindled variants of hairy cells have been described.

26. Distinctive features of B-PLL and T-PLL include predominance in elderly men, marked leukocytosis, generally homogeneous leukemic cells on blood smears, and massive splenomegaly.

27. Peripheralizing MCL should be excluded in cases of possible B-PLL, especially cases with CD5 coexpression; staining for cyclin D1 or FISH for CCDN/IgH is useful in confirming MCL.

28. Although a fair proportion of T-PLLs are morphologically similar to B-PLLs, other cases exhibit “knobby” nuclear irregularity with inconspicuous or absent nucleoli.

29. Cases of T-PLL can be identified by nuclear and cytoplasmic staining for TCL-1 or by cytogenetic detection of inv(14)(q11q32) or other T-PLL associated karyotype.

30. SMZL with villous lymphocytes by definition exhibits blood involvement at presentation; prominent clinical morphologic and immunophenotypic overlap between SMZL and HCL is evident.

31. The pattern of bone marrow infiltration can generally be used to distinguish SMZL from HCL; discrete nodules and intrasinusoidal lesions characterize SMZL, while HCL typically exhibits subtle, patchy interstitial infiltrates early in the disease course with progression to diffuse lesions in advanced cases.

32. Increased cytologically normal-appearing large granular lymphocytes are more common in T-cytotoxic/suppressor cell neoplasms, while true NK cell neoplasms are much less common and may exhibit greater cytologic immaturity and pleomorphism.
33. Because of morphologic and genetic overlap, cases formerly termed “T-chronic lymphocytic leukemia” are currently viewed as a subtype (small cell variant) of T-PLL.

34. Because ATLL is rare in nonendemic regions, other mature helper T-cell disorders such as T-PLL and Sézary syndrome (SS) should be systematically excluded.

35. The cerebriform appearance of Sézary cells in blood and bone marrow is best appreciated under high magnification, while the coarse nuclear lobulations of acute adult T-cell leukemia cells are more readily apparent on low magnification.

36. Bone marrow infiltrates by SS range from small, extremely subtle collections of cerebriform cells to frank lymphomatous infiltrates. Only the conspicuous lesions are linked to adverse outcome.

37. By periodic acid-Schiff stain on biopsy sections, subtle infiltrates of Sézary cells (periodic acid-Schiff negative) can be distinguished from diffusely positive myeloid precursor cells, but immunohistochemical detection is clearly the preferred modality to highlight occult infiltrates.

38. T-PLL, ATLL, and SS are all mature T-cell neoplasms that characteristically express CD4; both ATLL and SS are typically CD7−, while CD25 expression is more characteristic in ATLL.

39. Caution should be exercised in the flow cytometric assessment of blood for evidence of minimal involvement by SS, since low numbers of CD4+/CD7− T cells are physiologic.

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[22.10] References


