B-Lymphoblastic Leukemia in Patients With Chronic Lymphocytic Leukemia

A Report of Four Cases

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ABSTRACT

Objectives: B-lymphoblastic leukemia (B-LBL) arising in patients with chronic lymphocytic leukemia (CLL) is exceedingly rare and poorly characterized.

Methods: We describe four patients with CLL and concurrent or subsequent B-LBL diagnosed by morphologic, immunophenotypic, cytogenetic, and molecular analysis and reviewed the literature.

Results: In three patients, B-LBL followed CLL by 5 to 15 years, and in one patient, B-LBL was diagnosed simultaneously with CLL. In all cases, the CLL had a typical immunophenotype, and the B-LBL blasts showed an immature B-cell immunophenotype with expression of CD10, CD19, and TdT and absence of surface immunoglobulin. In two patients, B-LBL blasts harbored t(9;22)(q34;q11.2)/BCR-ABL1. We sequenced the IGHV genes in both CLL and B-LBL in two patients and showed that IGHV usage differed.

Conclusions: Our data suggest that at least some cases of B-LBL arising in patients with CLL are independent, secondary neoplasms rather than a manifestation of histologic transformation.
B-LBL is a neoplasm derived from progenitor B-lymphoid cells that usually involves bone marrow (BM), peripheral blood (PB), and less often lymph nodes. Patients most often have extensive BM and blood involvement, which results in pancytopenia. Lymphoblasts are typically small to medium in size with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli. B-LBL is more common in children, but B-LBL associated with t(9;22)(q34.1q11.2)/BCR-ABL1 (Philadelphia [Ph] chromosome) is more common in adults and associated with poorer outcome. Although lymphoblastic transformation of CLL has been previously reported, to our knowledge, no cases of Ph chromosome–positive B-LBL in patients with CLL have been described, and the clonal relationship between CLL and B-LBL has not been investigated.

In this study, we report four patients with CLL who subsequently developed or simultaneously had B-LBL. We describe the clinicopathologic, immunophenotypic, cytogenetic, and molecular findings for those four patients.

Materials and Methods

Case Selection

We searched the files of the Department of Hematology at The University of Texas MD Anderson Cancer Center for patients with both CLL and B-LBL, with material available for review, who were diagnosed between January 1991 and July 2014. Clinical and pathologic data were obtained by review of medical records. The study was performed with approval from the MD Anderson Internal Review Board.

Morphologic and Immunohistochemical Methods

May-Grünwald–Giemsa–stained PB smears were reviewed, and manual 100-cell differential counts were performed. Wright-Giemsa–stained BM aspirate smears and touch imprints were reviewed, and manual 500-cell differential counts were performed. BM aspirate, clot, and decalcified core biopsy specimens stained with H&E were also reviewed. Cytochemical analysis for myeloperoxidase was performed for patients using standard methods. Terminal deoxynucleotidyl transferase (TdT) was assessed on bone aspirate smears by using an anti-TdT antibody (Superblock, Bethesda, MD) and immunofluorescence microscopy (patients 1-3).

Immunohistochemical studies were performed on BM core biopsy or clot specimens. The panel of antibodies included reagents specific for CD3, CD5, CD10, CD34, CD79a, PAX5, and TdT. In situ hybridization analysis for Epstein-Barr virus was also performed on BM core biopsy specimens using a probe for Epstein-Barr virus–encoded RNA and a hybridization kit according to the manufacturer’s directions (Dako, Carpinteria, CA).

Flow Cytometry Immunophenotyping

Immunophenotypic analysis by flow cytometry was performed on BM aspirate or PB specimens from each patient. The specimens were processed by a standard RBC lysis method, initially viewed using CD45 vs side scatter and assessed by four-color flow cytometry as described previously. The panel of monoclonal antibodies included reagents specific for CD2, CD5, CD7, CD10, CD11c, CD13, CD14, CD19, CD20, CD22, CD23, CD33, CD34, CD38, CD45, CD64, CD79a, CD79b, CD117, HLA-DR, TdT, FMC7, and surface and cytoplasmic immunoglobulin k and l light chains. Ten thousand events were acquired using a FACSCalibur instrument (Becton Dickinson, San Jose, CA), and the data were analyzed using CellQuest software (Becton Dickinson).

Conventional Cytogenetics and Fluorescence In Situ Hybridization

Conventional cytogenetic analysis was performed on unstimulated and lipopolysaccharide-stimulated BM aspirate samples as described previously. Fluorescence in situ hybridization (FISH) was performed using an LSI-BCR/ABL ES dual-color translocation probe (Abbott Laboratories, Abbott Park, IL) to detect the presence of the BCR-ABL1 fusion gene as described previously.

Somatic Mutation Status of IGHV Genes

In two cases, the immunoglobulin heavy chain variable region (IGHV) genes were sequenced using total RNA extracted from BM aspirate or PB material as described previously. To determine the level of somatic mutation, we aligned sequences from patient samples with germline sequences in the V-BASE 2 database. IGHV status was categorized as unmutated if fewer than 2% of the sequences contained mutations (>98% homology to germline sequences) and as mutated if there were 2% or more mutations.

Quantitative Reverse-Transcription Polymerase Chain Reaction

BCR-ABL1 fusion transcripts were quantified in a multiplex TaqMan real-time reverse-transcription polymerase chain reaction (RT-PCR) assay that simultaneously detects b2a2, b3a2, and e12 transcript types as described previously. RNA was extracted from BM samples using Trizol reagent (Gibco-BRL, Gaithersburg, MD) according to the manufacturer’s instructions. Reverse transcription was performed on total RNA (1 µg) using random hexamers and Superscript II reverse transcriptase (Gibco-BRL). The
resulting complementary DNA was subjected to RT-PCR to amplify BCR-ABL1 fusion transcripts on an ABI PRISM 7700 Sequence Detector (PerkinElmer/Applied Biosystems, Foster City, CA) followed by capillary electrophoresis on an ABI PRISM 3700 Genetic Analyzer (PerkinElmer/Applied Biosystems) to determine transcript type. BCR-ABL1 levels were normalized to total ABL1 transcript levels.

**Results**

**Clinical Features**

Four patients formed the study group, and the clinical data are summarized in Table 1. There were two men and two women with a mean age of 59 years (range, 47-81 years) at the time of CLL diagnosis and a mean age of 67 years (range, 60-81 years) at the time of B-LBL diagnosis. Three patients (patients 1, 2, and 3) initially had CLL and developed B-LBL 5, 11, and 15 years later. Patients 2 and 3 at time of CLL diagnosis were treated with fludarabine, cyclophosphamide, and rituximab. In all three patients, residual CLL was also present at the time of B-LBL diagnosis. Patient 4 had concomitant CLL and B-LBL at the time of initial diagnosis.

Following the diagnosis of B-LBL, patients 1 and 2 were treated with rituximab with hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone. Patient 1 also received dasatinib, and patient 2 also received imatinib. Patient 4 was treated with vincristine, steroids, and doxorubicin. Treatment information was unavailable for patient 3. At the time of last follow-up, the disease in patient 1 remained in remission 24 months after diagnosis of B-LBL. In patient 2, the disease went into remission but relapsed 3 years later, and the patient died of the disease, 5 years after the initial diagnosis of B-LBL, after a second relapse while taking ponatinib. Patient 3 died 2 months after diagnosis of B-LBL. In patient 4, the disease went into remission, and the patient was placed on maintenance therapy with 6-mercaptopurine, vincristine, methotrexate, and prednisone but died of the disease 11 months after initial diagnosis of B-LBL.

**Morphologic and Immunophenotypic Findings at the Time of B-LBL Diagnosis**

At the time of B-LBL diagnosis, PB from patient 1 showed atypical lymphocytosis composed predominantly of CLL cells; however, a small number of lymphoblasts were identified. BM aspirate smears and touch preparations showed numerous lymphoblasts in a background of cells typical of CLL. The blasts were intermediate in size, with indented nuclei, fine chromatin, one or two small nucleoli, and scant cytoplasm. The BM biopsy specimen was hypercellular (100%) and packed with sheets of immature cells replacing almost the entire BM medullary space.

In patients 2 and 3, there was no absolute lymphocytosis in PB or morphologic evidence of CLL in BM, although flow cytometry immunophenotypic analysis showed evidence of minimal residual CLL. In both patients, BM was hypercellular (>95%) and extensively infiltrated by intermediate-sized blasts with fine chromatin, several small nucleoli, and scant cytoplasm.

Patient 4 was diagnosed with CLL and B-LBL simultaneously. A CBC count showed a hemoglobin level of 6.7 g/dL (normal range, 12.0-16.0 g/dL [120-160 g/L]), WBC count of 28.7 × 10^9/L (normal range, 4.11 × 10^9/L [4,000-28,700/μL]), and platelet count of 55 × 10^9/L (normal range, 150-440 × 10^9/L [150,000-440,000/μL]). A PB smear showed 64% small lymphocytes, consistent with CLL, and 35% lymphoblasts. BM was not examined in this patient.

Immunohistochemical analysis was performed on BM biopsy specimens of patients 1 and 3 and on a clot section of patient 2. For patient 1, the lymphoblasts were positive for CD10, CD34, CD79a, PAX5, and TdT and were negative for CD3 and CD5. For patient 3, lymphoblasts were positive for CD10, CD20, and TdT and negative for CD3 and CD117, and myeloperoxidase. The CD20-, CD10-, and TdT-positive cells were more than 70% of cellularity of the biopsy specimen in patient 3. For patient 2, more than 80% of cells on the clot section were positive for PAX5 and TdT.

Flow cytometry immunophenotypic analysis was performed on BM aspirate material (patients 1-3) or a PB specimen (patient 4) at the time of B-LBL diagnosis.

**Table 1**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at CLL Diagnosis, y</th>
<th>Age at B-LBL Diagnosis, y</th>
<th>Interval, y</th>
<th>Treatment for CLL</th>
<th>Treatment for B-LBL</th>
<th>Outcome (Follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>54</td>
<td>65</td>
<td>11</td>
<td>None</td>
<td>Hyper-CVAD, R, dasatinib</td>
<td>Alive without disease (24 mo)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>47</td>
<td>62</td>
<td>15</td>
<td>F, C, R</td>
<td>Hyper-CVAD, R, imatinib, ponatinib</td>
<td>Died with disease in second relapse (5 y)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>55</td>
<td>60</td>
<td>5</td>
<td>F, C, R</td>
<td>Unknown</td>
<td>Died 2 mo after diagnosis of B-LBL</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>81</td>
<td>81</td>
<td>0</td>
<td>None</td>
<td>Daunorubicin, steroid, vincristine</td>
<td>Died in 11 mo</td>
</tr>
</tbody>
</table>

B-LBL, B-lymphoblastic leukemia; C, cyclophosphamide; CLL, chronic lymphocytic leukemia; CVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone; F, fludarabine; R, rituximab.
Two populations of atypical B cells were detected in three patients (patients 1, 2, and 4). One population was CD45 strong positive and composed of monotypic small B-lymphocytes with an immunophenotype typical of CLL, including dim expression of surface immunoglobulin and CD20. The B cells were positive for CD5, CD19, CD20 dim, CD22, CD23, and dim monoclonal κ light chain in all four patients. A second population was CD45 dim positive and composed of intermediate-size cells with an immunophenotype of B-lymphoblasts (patients 1, 2, and 4), being positive for CD10 (all three patients), CD19 (all three patients), CD20 dim (patients 1 and 2), CD22 (all three patients), CD34 (patients 1 and 2), CD79a (all three patients), and TdT (all three patients). The lymphoblasts were negative for surface immunoglobulin and T-cell antigens. The BM aspirate specimen from patient 3 was suboptimal in quality with poor viability. Only B cells with an immunophenotype of CLL were detected.

Cytochemical analysis for myeloperoxidase was negative in the blasts in all three cases assessed (patients 1-3). In all three cases (patients 1-3) assessed for TdT by immunofluorescence microscopy, the blasts were positive. There were no discrepancies in TdT expression between the flow cytometry, immunofluorescence, and immunohistochemistry results in all three patients.

Cytogenetic and Molecular Results

The complete karyotype and FISH results for each patient are listed in Table 2. At the time of B-LBL diagnosis, t(9;22) was detected in patients 1 and 2 by conventional cytogenetics and FISH. For patient 1, initial chromosome analysis showed a diploid karyotype and FISH was not performed. At the time of diagnosis of B-LBL, the t(9;22) and BCR-ABL1 rearrangement were detected by conventional and FISH analyses Image 1H. FISH also revealed trisomy 12. For patient 2, conventional chromosome analysis was...
Image II (cont) The marrow was 100% cellular and composed of sheets of blasts with occasional intermixed smaller lymphocytes (H&E, ×500). The blasts were positive for CD10 (D) and PAX5 (E) (×400) and negative for CD5 (F) (×400). They were positive for terminal deoxynucleotidyl transferase by immunohistochemical analysis of the bone marrow biopsy specimen (G) (×400) and for BCR-ABL1 by fluorescence in situ hybridization analysis of a bone marrow aspirate smear (H) (×1,000).
not performed, and a FISH panel revealed del(13)(q14.3) at the time of CLL diagnosis. At the time of B-LBL diagnosis, conventional analysis showed a complex hyperdiploid clone, including t(9;22).

FISH was positive for BCR-ABL1 fusion, and a FISH panel was negative for del(13)(q14.3), which had been present at the time of initial CLL diagnosis. Patients 3 and 4 were negative by FISH for the presence of BCR-ABL1 fusion. Patient 3 had a diploid normal male karyotype at the time of CLL diagnosis and a complex hypodiploid clone at the time of B-LBL diagnosis.

RT-PCR analysis of BM or PB detected BCR-ABL1 fusion transcripts in two of three patients tested. Patients 1 and 2 had BCR-ABL1 fusion transcripts at the time of B-LBL diagnosis. An e1a2 BCR-ABL1 fusion transcript coding for a 190-kDa BCR-ABL1 fusion protein was detected in patient 1, and a b2a2 fusion transcript coding for the 210-kDa BCR-ABL1 fusion protein was detected in patient 2. No BCR-ABL1 fusion transcript was detected in patient 4.

Somatic mutation analysis of IGHV genes was performed on BM or PB specimens collected at the time of initial CLL diagnosis and at the time of B-LBL transformation for patients 1 and 2. For patient 1, both CLL and B-LBL BM samples showed no (0%) deviation from the germline sequences. However, neoplastic cells from the initial CLL sample used the V1H4-39 immunoglobulin family, and those from the B-LBL sample used the V1H3-23 family. For patient 2, IGHV mutation analysis performed at the time of CLL diagnosis showed a 5.2% deviation from the germline sequence and use of the V1H3-23 family, whereas at the time of B-LBL diagnosis, IGHV mutation analysis of a BM sample showed an unmutated IGHV gene (0.0% deviation) with use of V1H4-39.

Discussion

In this study, we describe four patients with CLL who developed B-LBL, a truly rare occurrence in patients with CLL. The four cases in this report represent 0.1% of all CLL cases observed at our institution over a 23-year period.

In an extensive literature review by Zarrabi et al in 1977, 31 patients with CLL who had acute leukemia were identified. Ten of these cases were considered acute lymphoblastic leukemia (ALL); these cases were not further characterized as B- vs T-cell lineage. Immunophenotypic and cytogenetic data were not included in the series. It is also worth emphasizing that a subset of the cases in the older literature probably would need to be confirmed as ALL. In recent decades, ancillary studies, not yet developed before 1977, have become an essential component of the classification of B-LBL, as outlined in the World Health Organization (WHO) classification system. A good example is a patient whose CLL was thought to have transformed into B-LBL18 and from whom the Z-138 cell line was derived. It is now recognized that that patient’s tumor had a complex karyotype with t(11;14)(q13;q32) and overexpressed cyclin D1, fulfilling current WHO criteria for blastic mantle cell lymphoma.19

Subsequent to the review by Zarrabi et al, only eight cases of B-LBL arising in patients with CLL have been reported. Three patients had B-LBL diagnosed within 2 months of CLL diagnosis; this short time interval likely represents concurrent CLL and B-LBL at initial presentation. The other five patients developed B-LBL 8 months to 7 years after the diagnosis of CLL. In some of the case reports with sufficient detail, the diagnosis of B-LBL is doubtful. For example, Torelli and colleagues11 described a patient with B-LBL who had L3 morphology according to the French-American-British classification system and who had disease positive for surface immunoglobulin. These findings, in retrospect, are more suggestive of Burkitt lymphoma in the leukemic phase.

Similarly, Miller et al20 reported a case of CLL presenting as a blast crisis; however, on the basis of their description of an immunoblastic lymphoma and detection of CD5 and surface immunoglobulin in the blasts, the diagnosis of DLBCL and Richter syndrome seems more likely.

Cytogenetic findings were reported for five of eight patients, but the Ph chromosome was not found by either conventional cytogenetic analysis or FISH analysis. All five patients had abnormal karyotypes with additional changes at the time of lymphoblastic transformation. For example, Mohamed et al6 reported 46,XY,+3,der(3)
In patients with Richter syndrome, in approximately 60% to 80% of patients the cells of CLL and DLBCL cases are clonally related.23-25 It also has been found that most cases of DLBCL clonally related to the precedent CLL arise from unmutated CLL. In contrast, in the remaining 20% to 40% of cases, the DLBCL is a clonally unrelated independent neoplasm. In these patients, the initial CLL is often mutated.24 Using microdissection and single-cell analysis, in some cases of the Hodgkin variant of Richter syndrome, the Reed-Sternberg and Hodgkin cells are clonally related to CLL, whereas others are not.25,26 Most of the Hodgkin variant of Richter syndrome occurs in patients with mutated CLL.25

The relationship between CLL and B-LBL has not been well studied. None of the cases reviewed by Zarrabi et al1 was studied for a clonal relationship between the CLL and ALL. Others (Table 3) have speculated that B-LBL arising in patients with Richter syndrome is clonally unrelated.22-24

Our findings based on the type of surface immunoglobulin or cytogenetic analysis; however, no cases were assessed for IGHV genes sequenced. The results from our analysis of paired CLL and B-LBL specimens from two patients with Ph chromosome–positive B-LBL showed in both cases that the CLL and B-LBL cells used different IGHV families and therefore were clonally unrelated. In one of these two patients, the IGHV gene was unmutated in both CLL and B-LBL cells; in the other patient, IGHV was mutated in CLL cells but not in B-LBL cells. These findings show that B-LBL can occur in a patient with either a mutated or unmutated IGHV.

In summary, we have described four patients with CLL who also developed B-LBL, including one patient with simultaneous CLL and B-LBL and three patients in whom B-LBL followed onset of CLL by 5 to 15 years. BCR-ABL fusion gene and transcripts were identified in two patients with B-LBL, with one having never been treated for CLL.

In aggregate, our data suggest that B-LBL in patients with CLL is an independent, secondary neoplasm rather than a manifestation of histologic transformation of CLL, at least in a substantial subset of patients. In addition, B-LBL can occur in patients with unmutated or mutated CLL.

References


