Diagnostic Problems Among Chronic Lymphocytic Leukemia and Other Indolent B-cell Leukemias in a Japanese Population

Yasushi Isobe¹, Junichi Tomomatsu¹, Yutaka Tsukune¹, Nobuhiro Tsukada², Makoto Sasaki¹, Koichi Sugimoto¹ and Norio Komatsu¹

Abstract

Objective Japanese chronic lymphocytic leukemia (CLL) provides a diagnostic dilemma due to the low incidence and the heterogeneity shown in its morphology and immunophenotype. We clarified the diagnostic problems in Japanese CLL through our retrospective observation.

Methods Between 2006 and 2011, we found a total of 48 cases with CLL and other indolent B-cell leukemias. We made a diagnosis of true CLL based on clinical, laboratory, immunophenotypic and cytogenetic data.

Results Among the 48 cases, only 28 cases (58.3%) were diagnosed with true CLL. Morphologic evaluation using a forced-air dried preparation alone is not helpful to distinguish CLL from other indolent B-cell leukemias, including hairy cell leukemia, mantle cell lymphoma, lymphoplasmacytic lymphoma, and splenic marginal zone lymphoma. CLL immunophenotypic score should be more strictly applied in Japan than in Western countries.

Conclusion Fluorescence in situ hybridization for CCND1/IGH, the presence of leukocytosis and lymphadenopathy at diagnosis, and the morphological evaluation using naturally air dried preparations are important clues to make a correct diagnosis of Japanese CLL.

Key words: chronic lymphocytic leukemia, CD5, Japanese patients, naturally air-dried preparation, fluorescence in situ hybridization


Introduction

Chronic lymphocytic leukemia (CLL) is a mature B-cell neoplasm characterized by the expansion of mature small- to medium-sized lymphocytes in the peripheral blood and other common manifestations, such as lymphadenopathy, splenomegaly, and hepatomegaly (1, 2). Most leukemic cells show a scant cytoplasm and a dense nucleus lacking discernible nucleoli, and are clearly positive for CD5 and CD19 (1-3). The expression levels of surface immunoglobulin, CD22 and CD79b are dim, and the CD20 expression level is low compared with that detected in normal B cells (1-4). Although CLL is the most common type of adult leukemia in Western countries, this disease is rare in Japan (5-7). The incidence in Japan has been reported to be below 0.48 per 100,000-person years, and it is lower than that of mantle cell lymphoma (MCL) (5, 6). We recently reported 28 Japanese CLL cases showing three morphologic variants, diverse immunophenotypic features, and frequent somatic hypermutation in immunoglobulin heavy chain gene (8). Indeed, we found that many Japanese cases presented with a favorable clinical course compared with typical Western CLL (8). Due to the uniform features in Western countries, morphologic and immunophenotypic analysis are reliable to define CLL among other B-cell leukemias, including MCL, hairy cell leukemia.
leukemia (HCL), prolymphocytic leukemia (PLL), lymphoplasmacytic lymphoma (LPL), and splenic marginal zone lymphoma (SMZL) (1-4). These diseases have different clinical behaviors from that of CLL. Treatment strategies are also distinct in each disease entity. In this paper, we show several practical clues for making the correct diagnosis of Japanese CLL.

Materials and Methods

Patients

Patients with indolent B-cell leukemia, who attended Juntendo University Hospital between November 2006 and May 2011, were candidates for this retrospective observation. Lymphoma cases without leukemic conversion were not included. A biopsy and further evaluation were performed after obtaining written informed consent from each patient in accordance with the Declaration of Helsinki. All patients presented with clonal B-cell proliferation in peripheral blood, which was confirmed by flow cytometry (κ/λ, ratio of <1:1 or ≥2:1:1) or Southern blot analysis of immunoglobulin JH gene rearrangement.

Diagnosis

Although diagnosis of indolent B-cell leukemias was made according to the WHO classification, CLL was made by the original criteria (8). That is as follows: 1) persistent lymphocytosis of more than 5.0×10⁹/L lasting at least for three months; 2) morphologically small- to medium-sized lymphocytes with or without nucleoli; 3) morphologically no hair formation of cell membrane in naturally dried preparation; 4) CD5-positive, CD19-positive and CD10-negative immunophenotypes; 5) lack of t(11;14)(q13;q32) or cyclin D1 translocation; 4) CD5-positive, CD19-positive and CD10-negative immunophenotypes; 5) lack of t(11;14)(q13;q32) or cyclin D1 immunostaining.

Clinical data, morphological evaluation and fluorescence in situ hybridization analysis

We reviewed a total of 48 cases. Patients’ history, physical findings, laboratory, and imaging data were collected. Although peripheral blood smears are usually desiccated only by forced-air drying in Japan, we performed two different methods, i.e., forced and natural air drying. In the natural air-drying procedure, each smear preparation is left at room temperature at least for three hours until it is dried. Although the conventional forced-air drying procedure is advantageous to detect cytoplasmic structures of cells, we beneficially recognize hair formation of leukemic-cell membrane and the presence of nucleoli using natural air-dried preparations. Each sample was stained using the Wright-Giemsa method and evaluated by microscopic study. To exclude follicular lymphoma (FL) and MCL, we performed fluorescence in situ hybridization (FISH) analysis for BCL2-IGH and CCND1-IGH translocations in CD10-positive and CD5-positive cases, respectively.

Evaluation of cell surface antigens

Cell surface antigens were detected by flow cytometry (FCM). Fluorescein isothiocyanate- or phycoerythrin-conjugated antibodies were used for the detection of surface antigens, including CD3, CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD27, CD38, CD103, α, μ, γ, κ, λ, and FMC7. Each sample was run with the negative control stained with appropriate isotype-matched control antibodies.

Morphologic aspects of CLL and other chronic B-cell leukemias

In typical CLL cases, forcedly air-dried preparations of a peripheral blood showed constant medium-sized lymphocytes with scanty cytoplasm and airbrush colored-appearing chromatin. Nuclear margin adhered to the inside of cell membrane at one or two sites. Nucleoli were not seen in typical CLL cells (Fig. 1). In contrast, natural air-dried preparations clarified that leukemic cells were slightly smaller than those observed using forced-air drying, and had little cytoplasm and condensed nuclear chromatin. In this situation, cytoplasm is poor, and the outline is regular without hair formation (Fig. 1). These alterations may be influenced by the difference in dehydrated conditions of each cytology. Leukemic conversion of follicular lymphoma (FL) had morphologically similar characteristics to typical CLL. The present two cases had smudge cells. Leukemic FL cells were slightly smaller and had less cytoplasm than those of CLL in both desiccating protocols. Nuclear chromatin was quite monotonous compared with the pattern of CLL (Fig. 1). Leukemic MCL was indistinguishable from CLL even by morphological evaluation (Fig. 1). Analyses for variation in cell size, indented nucleus, and increased proportion of prolymphocytoid cells are helpful to identify MCL.

We previously described two morphological variants such as prolymphocytoid (Pro)- and lymphoplasmacytoid (LP)-types (8). Pro-type CLL cells usually possess one or two nucleoli and are morphologically similar to prolymphocytes. These features are shared in common among SMZL, HCL and variant type of HCL (HCLv). In forced-air dried preparations, these leukemic cells had abundant cytoplasm, and some of the cells presented with ‘sunny-side up’ morphology (SMZL in Fig. 1). Although this finding was also observed in Pro-type CLL, morphological evaluation using natural air-dried preparations clearly detected hair formation in HCL, HCLv, and SMZL but not in Pro-type CLL. LP-type CLL is characterized by nuclear irregularity, less condensed chromatin in forced-air dried preparation and having Golgi zone adjacent to the nucleus in natural air-dried preparations. Indeed, these morphologic features were similar to those of LPL (Fig. 1). Although this variant showed a gray zone between CLL and CD5-positive LPL, we consid-
Figure 1. Morphological aspects of indolent B-cell leukemias. Peripheral blood smears were desiccated using two methods, i.e., forced (dryer-used) and natural air drying. Morphologically, it is difficult to distinguish among chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL) and leukemic conversion of follicular lymphoma (FL). Some of splenic marginal zone lymphoma (SMZL) cells showed ‘sunny-side up’ morphology in forced air-dried preparations and hair formation in natural air-dried preparations. Lymphoplasmacytic lymphoma (LPL) cells are characterized by nuclear irregularity, less condensed chromatin and having Golgi zone adjacent to the nucleus.

Immunophenotypic features of CD5-positive B-cell leukemias

The CLL score (5) is listed in Table 1. Typical immunophenotypes of CLL (3 points and above) were observed in 17 of 19 examined cases showing a typical morphology (89.5%). We previously showed Pro-type CLL and CD5-positive SMZL cells equally expressed CD20 and varied their expression levels of CD5, CD23, FMC7 and light chains (8). Because CD5-positive SMZL and Pro-type CLL similarly showed two or three points (Table 1), we failed to identify these two diseases by the CLL score. In LP-type CLL, expression levels of CD20 and light chains were relatively high, and all three cases were negative for CD23. The scoring gave two or three points (Table 1). Thus, these morphologic variants showed a relatively low score and are im-

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Among them, three cases were diagnosed with leukemic FL cells. In all the three leukemic cases, five cases with monoclonal gammopathy (two IgM, one IgG and one IgA types) were diagnosed with LPL because of lymphoplasmacytoid lesion in the bone marrow. These cases showed a lymphocyte count of less than 5.0×10⁹/L at diagnosis. Four cases showing hair formation on the leukemic-cell surfaces were diagnosed with HCL (1 case), HCLv (1 case) and SMZL (2 cases) by their immunophenotypic profiles. The HCL case did not show a lymphocyte count of more than 5.0×10⁹/L during the observation period.

In contrast, CD5-positive mature B-cell expansion was observed in 36 cases (75.0%). They presented with a lymphocyte count of more than 5.0×10⁹/L at diagnosis. FISH analysis of CCND1-IGH and immunohistochemical detection of cyclin D1 disclosed four cases with MCL (11.1%). During the diagnostic process, the most difficult problem was to distinguish CD5-positive SMZL from CLL. We found the two helpful findings (i.e., the presence of splenomegaly without lymphadenopathy at diagnosis and the detection of hair formation in natural air-dried preparations) to identify SMZL in cases with CD5-positive B-cell leukemias. On the basis of these points, four SMZL cases were diagnosed (11.1%). Based on clinical and laboratory data, we finally identified 28 Japanese CLL among 48 cases with indolent B-cell leukemias (58.3%). Thus, above 40% of the cases were not diagnosed with CLL.

### Table 1. CLL Score in 36 Cases with CD5-positive B-cell Leukemia

<table>
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<tr>
<th>CLL score*</th>
<th>typical CLL** (n = 22)</th>
<th>Pro-type CLL (n = 3)</th>
<th>LP-type CLL (n = 3)</th>
<th>MCL (n = 4)</th>
<th>SMZL (n = 4)</th>
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* The scoring gives one point when the marker is typical for CLL, i.e., CD5 positive, CD23 positive, FMC7 negative, surface immunoglobulin dim and CD22 dim/negative [4].

** Among CLL cases with typical morphology, one CLL/PLL case is included.

Abbreviation. CLL: chronic lymphocytic leukemia, Pro-type: prolymphocytoid type, LP-type: lymphoplasmacytoid type, MCL: mantle cell lymphoma, SMZL: splenic marginal zone lymphoma, NA: not available

### Diagnostic process of Japanese CLL

The diagnostic process is shown in Fig. 2. We found a total of 48 cases with indolent B-cell leukemia at our institution. The first step of CLL diagnosis is to detect CD5 on the leukemic cells using FCM. Indeed, CD5-negative mature B-cell clonal expansion was observed in 12 of the 48 cases (25.0%). Among them, three cases were diagnosed with leukemic FL because of the presence of IGH-BCL2 translocation. We noted that the expression level of CD10 on leukemic FL cells was dim in all the three leukemic cases. Five cases with monoclonal gammopathy (two IgM, one IgG and one IgA types) were diagnosed with LPL because of lymphoplasmacytoid lesion in the bone marrow. These cases showed a lymphocyte count of less than 5.0×10⁹/L at diagnosis. Four cases showing hair formation on the leukemic-cell surfaces were diagnosed with HCL (1 case), HCLv (1 case) and SMZL (2 cases) by their immunophenotypic profiles. The HCL case did not show a lymphocyte count of more than 5.0×10⁹/L during the observation period.

Compared with CLL from Western countries, we found that Japanese CLL showed heterogeneity in its morphology and immunophenotype. Because the incidence of CLL in Japan is estimated to be below one-tenth of that in Western countries (5-7), it is important to collect the definite cases for evaluation of Japanese CLL for clinical studies. Although morphological evaluation alone failed to exclude other indolent B-cell leukemias, we found two important findings, i.e., ‘sunny-side up’ morphology in forced-air dried preparations and hair formation shown only in natural air-
dried preparations. These findings were suggestive of HCL, HCLv and SMZL. We believe that two desiccating protocols for peripheral blood smear are useful for morphological evaluation of indolent B-cell leukemias.

FCM analysis of leukemic cells is the 'gold-standard' method for diagnosis of CLL and other indolent B-cell leukemias (1-4). CD5 is recognized as an essential marker for diagnosis of CLL (1-3). CD5-negative indolent B-cell leukemias are rare and seem to include heterogeneous diseases (9). Our observation suggests that they should include FL, HCL, HCLv, SMZL and LPL. Moreover, they can possibly include CD5-negative MCL and leukemic conversion of NMZL. In the present study, we found that FL and MCL cases with lymphocytosis are morphologically indistinguishable from CLL. Indeed, it was difficult to confirm CD10 expression using FCM in leukemic FL cases. By using only immunohistochemistry detection of cyclin D1, HCL may be misdiagnosed as MCL because most HCL cases are weakly positive for cyclin D1 (10). In addition to morphological and immunophenotypic assessment, it is important for the correct diagnosis to perform FISH analysis regarding BCL2-IGH and CCND1-IGH translocations in these cases. Thus, we assume that CD5-negative CLL may be extremely rare. An important marker for CLL diagnosis, CD5 is occasionally detected in other indolent B-cell lymphomas, including SMZL, LPL and mucosa-associated lymphoid tissue lymphoma other than CLL and MCL (2, 11-14). To exclude these diseases, we adopted the CLL score for the present cases (4). Reflecting the presence of morphological variants, some CLL cases showed atypical results. The CLL score did not distinguish Pro- and LP-type variants from CD5-positive SMZL and LPL, respectively. The present results indicate that about one-fifth of diagnosed Japanese CLL may correspond to immunophenotypically atypical CLL, which is usually excluded in clinical trials performed in Western countries. CLL cells show no hair formation on the cell surface, and SMZL hardly show lymphadenopathy without splenomegaly at diagnosis. Therefore, we emphasize the importance to confirm the correct clinical information at diagnosis in addition to morphological and immunophenotypic results.

In conclusion, typical CLL, which is frequently observed in Western countries, is rare and accounts for about 50% of indolent B-cell leukemias in a Japanese population. In addition to Japanese routine work-up, morphological evaluation using natural air-dried preparations is essential for diagnosis of CLL. It is important to recognize the existence of grey zone leukemia showing continuity with LPL and SMZL. To elucidate these points, further accumulation of Japanese CLL cases is needed.

The authors state that they have no Conflict of Interest (COI).

References