Original Article

Clinical significance of flow cytometric evaluation of bone marrow involvement in B-cell lymphoma

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Bone marrow of 85 patients with B-cell lymphomas at initial presentation or at relapse was examined simultaneously using both two-color flow cytometry with a CD 19 gate (FCM) and pathologic examination (PTH) including bone marrow aspiration with or without core biopsy. Diffuse large-cell lymphoma (29 patients) and follicular lymphoma (20 patients) were two major subtypes. CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone)-based regimens were most frequently used to 47 patients. The median follow-up was 10.5 months. The estimated 2-year overall survival (OS) of the FCM- and PTH-negative group (49 patients), the FCM-positive but PTH-negative group (23 patients) and the FCM- and PTH-positive group (13 patients) was 69± 7 %, 45±11% and 31±15%, respectively. The estimated 2-year progression-free survival (PFS) of the first, second and third group was 69± 7 %, 30±11% and 21±13%, respectively. These results suggest that FCM together with PTH predict the prognosis of B-cell lymphoma. Prospective studies are needed to define the role of flow cytometirc identification of bone marrow lymphoma involvement.

(Key words : Lymphoma, Bone marrow, Flow cytometry)

Introduction

B-cell lymphoma is derived from B-lymphocytes at various stages of differentiation. Although it shows heterogenicity with different histologic and clinical features, most cells express B-cell antigens such as CD 19 and CD 20[1]. Routine staging in B-cell lymphoma includes evaluation of bone marrow involvement, because bone marrow infiltration affects both prognosis and treatment strategies[2,3]. Usually bone marrow assessment is performed by PTH using bone marrow biopsy and aspiration[4]. Previously we established a new flow cytometric method, CD 19-gating flow cytometry[5]. This two-color flow cytometry specifically detects B-cells in the area of CD 19-positive cells. We performed the mixing experiments of B-cell lymphoma cells

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with normal bone marrow mononuclear cells ; the CD 19-gating flow cytometry enables at least detection of 10% or more clonal B-cells existing in the bone marrow mononuclear cells[5]. Since mononuclear cells correspond to about one-fifth of whole bone marrow cells, this flow cytometry detects about 2 % of clonal B-cells in whole bone marrow cells. We and others have shown that flow cytometry is a powerful tool to detect occult B-cell lymphoma infiltrating the bone marrow and that this method is generally more sensitive than PTH[5-8]. In our knowledge, there is only one report on the association between flow cytometric detection of B-cell lymphoma cells in the bone marrow and prognosis[9]. Therefore, we examined whether the identification of bone marrow involvement in B-cell lymphoma using our flow cytometric method can predict clinical outcome.

Patients and Methods

Between December 1996 and February 2003, 86 patients with B-cell lymphoma who were admitted to Jichi Medical School Hospital at initial presentation or at relapse were retrospectively analyzed (Table 1). One patient with B-cell lymphoma was excluded because bone marrow examination showed FCM-negativity but PTH-positivity. Histological subtypes were mainly defined according to the International Working Formulation. All patients were diagnosed as having B-cell lymphoma with CD 19 using single-color FCM. B-cell lineage was also confirmed using immunostaining in most patients. Clinical staging at initial presentation or at relapse was done according to the Ann Arbor Classification. Both flow cytometric

Table 1 Characteristics of the patients

	FCM(-)PTH(-) (n=49)	FCM(+)PTH(-) (n=23)	FCM(+)PTH(+) (n=13)	*P*
Age (yr)				0.8728
Mean	61.5	61.3	59.3	
Gender (no)				0.1169
Male	26	13	11	
Female	23	10	2	
Disease (no)				0.3801
Initial	41	20	9	
Relapse	8	3	4	
Performance status (no)				0.2211
0 or 1	27	10	4	
≧2	22	13	9	
Histology (no)				0.5714
Diffuse large	14	10	5	
Follicular	12	6	2	
MALT	6	1	1	
Mantle	2	3	1	
Others	15	3	4	
Stage (no)				0.0016
I or II	21	3	0	
III or IV	28	20	13	
Therapy (no)				0.0897
CHOP-based	22	15	10	
Others	27	8	3	

FCM, flow cytometry with a CD 19 gate for bone marrow ; PTH, pathologic examination for bone marrow ; (+), positive result ; (-), negative result.

analysis of bone marrow cells and bone marrow core needle biopsy and/or aspiration were simultaneously performed in each patient before therapy. Therapy was selected based on International Prognostic Index, age, lymphoma histology, clinical stage, performance status and disease presentation. Flow cytometric results on bone marrow were never incorporated into the decision-making of therapy.

FCM was used for evaluation of lymphoma cells infiltrating the bone marrow as described previously[5]. Briefly, bone marrow mononuclear cells were dually stained with fluorescent isothiocyanate-conjugated goat anti-human immunoglobulin (Biosource International, Camarillo, CA), anti-human kappa light chain (Biosource) or anti-human lambda light chain (Biosource) and phycoerythrin-conjugated CD 19 (B4 ; Coulter Immunology, Hialeah, FL). Samples were collected in side scatter versus CD 19 histogram with gating on a B-cell cluster. The clonality of B-cells was determined by quantitation of kappa and lambda light chain expression by CD 19-positive cells. Normal kappa/lambda ratios are within the range of 0.5 to 3.0. Values outside this range were regarded as indicating the existence of clonal B-cells.

Association of flow cytometric analysis of bone marrow with clinical parameters was assessed by the χ^2 test. Survival probabilities were calculated with the product limit method according to Kaplan-Meier[10]. OS was calculated from the date of FCM to the date of death or last follow-up evaluation. PFS was calculated from the date of FCM to the date of progression, death or last follow-up evaluation. Differences between survival curves were analyzed by the log-rank test. In univariate analysis for OS and PFS, a proportional hazards model[11] was used for the following variables : age, gender, disease presentation, performance status, clinical stage, bone marrow involvement evaluated by PTH and bone marrow involvement evaluated by FCM.

Results

Patient characteristics are shown in Table 1. According to flow cytometric results, three groups were distinguished : the FCM- and PTH-negative group, the FCM-positive but PTH-negative group, and the group in which both tests were positive. Among these three groups, there were no differences in age, gender, disease presentation, performance status, histology or therapy. The major histologic subtype among them was diffuse large-cell lymphoma. The percentage of advanced stages, i.e., stages III and IV, was 57% in the FCM- and PTH-negative group and 87% in the FCM-positive but PTH-negative group. In clinical stage III or IV without bone marrow infiltration evaluated by PTH, FCM-positive patients accounted for 42% (20 patients/48 patients). CHOP-based regimes including CHOP, modified dose of CHOP, and CHOP with anti-CD 20 or irradiation were frequently used in each group. Other treatments included COP (cyclophosphamide, vincristine and prednisolone)-based regimens, Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, prednisolone, methotrexate and cytarabine), irradiation, watch and wait, and so on.

The median follow-up was 10.5 months. The projected OS in three groups was shown in Figure 1 : the most favorable was the FCM- and PTH-negative group, the intermediate was the FCM-positive but PTH-negative group, and the worst was when both were positive (p=

0.0270). When the FCM-positive and FCM-negative groups were separated, the former showed a significantly worse OS, compared to the latter (p=0.0197). Similar results on the projective PFS among three groups were obtained (Figure 2 ; p=0.0016). The FCM-positive group showed a significantly worse PFS, compared to the FCM-negative group (p=0.0007). Since diffuse large-cell lymphoma was the major subtype in each group, OS and PFS in this subtype were analyzed (Figure 3) : OS as well as PFS in the FCM-positive group was worse than that in the FCM-negative group, although the difference was not significant (p=0.1814 and p=0.1101, respectively). In follicular lymphoma, OS and PFS were similar between the FCM-negative group and FCM-positive group (p=0.7422 and 0.7841, respectively).

When all of the lymphomas were divided into the PTH-negative group and the PTH-positive group, the former showed better OS and PFS than the latter (p=0.0206 and p=0.0120, respectively). In the FCM-positive but PTH-negative group, three patients were in clinical stage I or II (Table 1). One patient with follicular lymphoma received involved field irradiation. About 16 months later from the end of the irradiation, new enlarged lymph nodes from mediastinal to para-aortic regions were detected. She was transferred to annother hospital to receive chemotherapy. The second with mantle cell lymphoma, who received several intensive

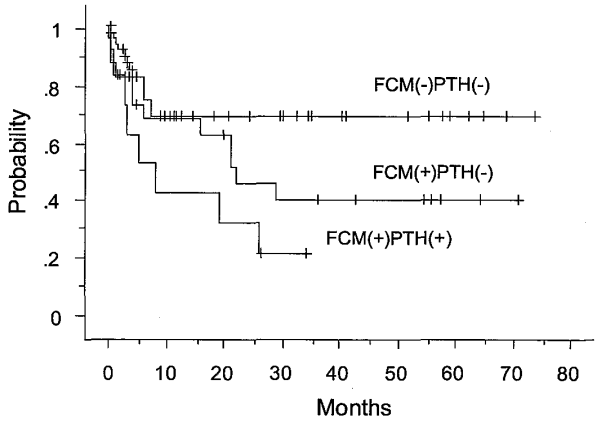

Figure 1 OS in B-cell lymphoma among three groups. FCM, flow cytometry with a CD 19 gate for bone marrow : PTH, pathologic examination for bone marrow ; (+), positive result ; (−), negative result.

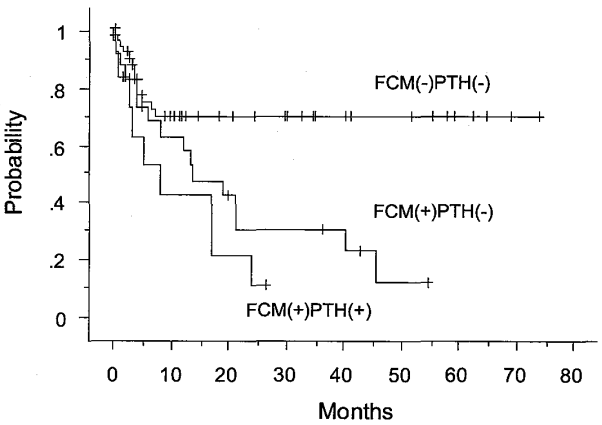

Figure 2 PFS in B-cell lymphoma among three groups. FCM, flow cytometry with a CD 19 gate for bone marrow ; PTH, pathologic examination for bone marrow ; (+), positive result ; (−), negative result.

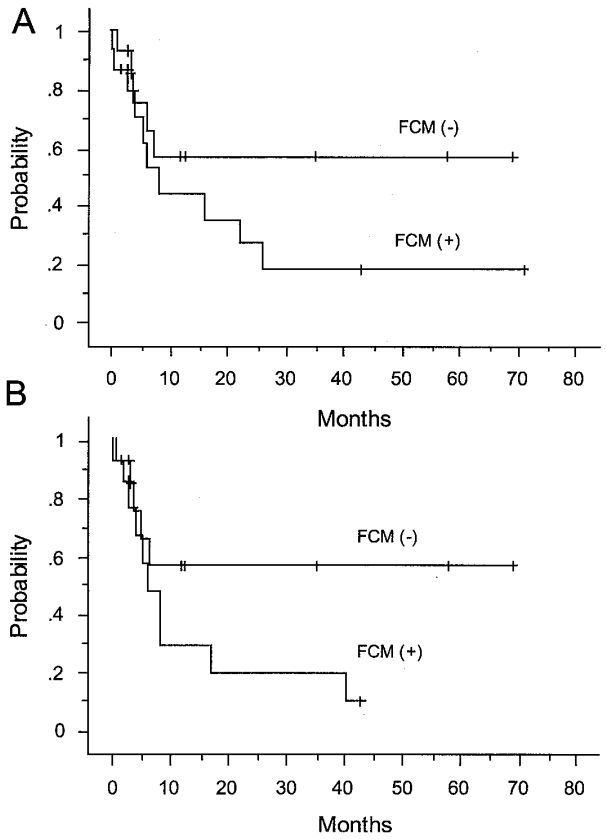

Figure 3 OS (A) and PFS (B) in diffuse large-cell lymphoma. FCM, flow cytometry with a CD 19 gate for bone marrow; PTH, pathologic examination for bone marrow ; (+), positive result ; (−), negative result.

chemothrapeutic regimens, died because of peritoneal lymphoma. The third with diffuse large-cell lymphoma was lost to follow-up after the surgical removal of the affected lymph node.

The parameters in OS were analyzed using the univariate analysis : old age (>65 years), poor performance status (II or III or IV), advanced stage (3 or 4), bone marrow involvement evaluated by PTH, and bone marrow involvement evaluated by FCM were poor prognostic factors. Similar results of poor prognostic factors on PFS except old age were obtained.

Discussion

In the management of patients with lymphoma, evaluation of prognostic factors is necessary ; factors that have generally been accepted as being associated with a poor prognosis are old age, poor performance status, B symptoms, disseminated stage, abdominal mass, extranodal sites of disease, and bone marrow involvement[3]. Several methods to predict a clinical outcome in lymphoma have been reported : minimal involvement by lymphoma identified by culturing lymphoma cells from morphologically negative marrow is statistically associated with a poor clinical outcome[12]. Detection of minimal disease in bone marrow in patients with follicular lymphoma by polymerase chain reaction analysis for bcl-2 translocation is associated with a poor clinical outcome, although the clinical significance of bcl-2/IgH rearrangement in follicular lymphoma remains controversial[13,14]. Eradication of polymerase chain reaction detectable immunoglobulin gene rearrangement of bone marrow in B-cell lymphoma is associated with decreased relapse[15].

In this study, we assessed the clinical relevance of bone marrow involvement evaluation in B-cell lymphoma using FCM before therapy. The flow cytometric identification of bone marrow lymphoma involvement was associated with poor clinical outcomes. In a routine practice, bone marrow infiltration of lymphoma is examined using bone marrow biopsy and aspiration. Taken together, we suggest that FCM concurrently with PTH should be performed to evaluate occult B-cell lymphoma infiltrating the bone marrow. Generally, flow cytometry shows superior sensitivity to PTH and results from flow cytometry are rapidly obtained[5-8]. We do not know whether FCM is able to completely substitute PTH in the detection of occult B-cell lymphoma. PTH has specific abilities in the survey of bone marrow such as evaluation of bone marrow cellularity and bone marrow fibrosis. Therefore, we believe that these two methods are essential for diagnosing bone marrow involvement of B-cell lymphoma.

B-cell lymphoma is a heterogeneous lymphoproliferative disorder : it includes indolent lymphoma and aggressive lymphoma. The International Prognostic Index for aggressive lymphoma including diffuse large-cell lymphoma at initial diagnosis and at relapse has been described[16]. Five parameters independently contribute to the prognosis as follows : age, stage, number of extranodal sites, performance status, and serum lactate dehydrogenase level. The subdivision of patients according to the number of prognostic factors into low risk (0 or 1 factor), low-intermediate risk (2 factors), high-intermediate risk (3 factors), or high risk (4 or 5 factors) with predicted 5-year OS values of 73%, 51%, 43%, and 26%, respectively[17]. Since bone marrow involvement is considered as one of the extranodal sites, it is important to estimate IPI in diffuse large-cell lymphoma by bone marrow examination. In our study, flow cytometric identification of bone marrow involvement in diffuse large-cell lymphoma was not statistically associated with a clinical outcome. However, the number of analyzed patients with the disease was small, the follow-up time was short, and a uniform regimen was not given. Therefore, it is not possible to draw a definitive conclusion from our study.

Recently, an international group proposed a prognostic index for follicular lymphoma, the Follicular Lymphoma International Prognostic Index (FLIPI)[18]. Five prognostic factors were selected : age, Ann Arbor stage, hemoglobin level, number of nodal areas, and serum LDH level. Three risk groups were defined : low risk (0-1 factor), intermediate risk (2 factors), and poor risk (3 factors) with predicted 10-year OS values of 71%, 51%, and 36%, respectively[18]. Since bone marrow involvement is included in Ann Arbor stage, it is necessary to perform bone marrow examination in follicular lymphoma. In our study, flow cytometric identification of bone marrow involvement in follicular lymphoma was not statistically associated with a clinical outcome. These results may be due to small number of patients with the disease and heterogeneous treatments.

Further studies are needed to determine the role of flow cytometric identification of bone marrow lymphoma focusing on both a distinct histologic subtype and a uniform regimen.

Acknowledgements

This study was supported by Jichi Medical University Young Investigator Award.

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フローサイトメトリーを用いたB細胞性リンパ腫における骨髄浸潤評価の臨床的意義

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要　約

初発または再発の85人のB細胞性リンパ腫患者の骨髄を，2重染色とCD19ゲート法を用いたフローサイトメトリー検査（FCM）と骨髄穿刺または骨髄生検による病理組織学的検査（PTH）を同時行った。B細胞性リンパ腫の主な組織型は，びまん性大細胞型リンパ腫(29例)と濾胞性リンパ腫（20例）であった。最も頻回に行われた治療法はCHOP療法を基礎にした化学療法で47例の患者に行われた。平均観察期間は10.5ヶ月であった。2年の生存率は，FCM陰性PTH陰性（49例）で69±7%，FCM陽性PTH陰性(23例)で45±11%，FCM陽性PTH陽性（13例）で31±15%であった。2年の無病生存率は，FCM陰性PTH陰性で69±7%，FCM陽性PTH陰性で30±11%，FCM陽性PTH陽性で21±13%であった。PTHとともに行うFCMは，B細胞性リンパ腫の予後の指標となることが示唆された。FCMを用いて悪性リンパ腫の骨髄浸潤を評価する意義を確認するためには，前方向試験が必要である。

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